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ON THE VALUE OF THE "ABORTIN" AS A DIAGNOSTIC AGENT FOR INFECTIOUS ABORTION IN CATTLE.*

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During his experiments on infectious abortion, Bang¹ observed that artificially infected animals, following a subcutaneous injection of a serum broth culture, reacted with a marked febrile rise of temperature, associated with inappetency and slight diarrhea. Based on another experiment, conducted on a healthy bull calf, which also reacted to an injection of the same material, the investigator concluded that the febrile reaction has no specific diagnostic value. Unfortunately Bang has not yet reported the experiments he intended to conduct with sterile cultures.

Recently the English Commission, M'Fadyean and Stockman,² mentioned, in their report on epizootic abortion, noteworthy results with the glycerin extract of *B. abortus*, it being their intention to prepare a diagnostic agent similar to those known as tuberculin and

* Received for publication July 7, 1913.

¹ *Ztschr. f. Tiermed.*, 1897, 1, p. 241; *Archiv. f. Wissenschaft und Prakt. Tierheilk.*, 1907, 33, p. 312.

² *Report of Departmental Committee on Epizootic Abortion*, Part I, "Epizootic Abortion in Cattle," and Appendix to Part I, "Epizootic Abortion in Cattle," London, 1909; *Jour. Comp. Path. and Therap.*, 1910, 23, p. 370.

mallein. The biological product they obtained was called by them "abortin." The technic of its preparation was given as follows:

A 1 per cent glucose serum glycerin broth, infected with *B. abortus*, was grown for 6 weeks at 37° C., sterilized for 2 hours at 99° C., then filtered through filter paper or Berkefeld candles, and 1 per cent carbolic acid added, or, if not used immediately, concentrated on the water bath to one-tenth of its volume.

This preparation, injected subcutaneously or intravenously, produced in infected animals a rise of temperature which began about the fourth hour and lasted about 14 hours. The intravenous injection was frequently accompanied by rather alarming general symptoms, similar to those of an anaphylactic shock. The subcutaneous injection with 0.5 to 1 c.c. "abortin brute" was, however, harmless. In normal animals no reactions were recorded. Animals which had aborted as a sequel to the infection with *B. abortus* still reacted several months afterward. Basing their conclusions on their results, the English Commission considers the "abortin reaction" to be a specific one. This publication has been the subject of considerable comment, and in various laboratories an attempt was made to confirm or to deny the diagnostic value of "abortin."

Belfanti,¹ in comparing the value of a few diagnostic agents for infectious abortion, came to the conclusion that the "abortin" should be considered as an unreliable diagnostic agent until contrary proofs are given. He doubts the specificity of the reaction. From his publication the following results are interesting:

Out of 19 animals tested, 10 reacted to the "abortin"; only 7 were positive to the serum test; 6 did not react to the "abortin," yet only 3 were negative to the serum test. Three doubtful "abortin" reactions belong to the group of 9 animals which were doubtful to the serum tests.

The preparation he used came from the laboratories of Jensen and Zwick, and his own make. Zwick and Zeller,² in their experiments with "abortin," reached the conclusion that the "abortin" is not suited as a diagnostic for the determination of infectious abortion. Frequently the "abortin" reactions did not correspond with the serum reactions. Their publication is largely responsible for our own work, and there will be later opportunities offered to

¹ *Ztschr. f. Infektionskr., d. Haustiere*, 1912, 12, p. 1.

² *Arb. a. d. k. Gsndtsamte.*, 1912, 43, p. 95.

discuss some of their results in connection with ours. Holth¹ expresses the opinion that possibly the "abortin" will be a useful, perhaps more delicate, reagent for the determination of early stages of the abortus infection than the usual, reliable immunity reactions. Caspar,² Brüll,³ DeVine,⁴ and others report, without going into detailed account of their work, only unreliable results. Mohler and Traum⁵ in this country report failures. Giltner,⁶ in his work on "abortin," has, unfortunately, carried out no serum tests, and, therefore, his results are not suitable for comparison.

In general these results were not as claimed by M'Fadyean and Stockman, and, as it was our intention to form our own independent idea as to the action and importance of the "abortin" as a diagnostic agent, a series of experiments was undertaken. This work was readily possible, as the laboratory for the last two and one-half years has been engaged in a detailed study of infectious abortion of cattle. Furthermore, as inquiries were constantly received regarding the value of the "abortin" which is at present commercially distributed in this country, a most thorough investigation was urgently demanded.

THE PREPARATION OF "ABORTIN."

Different culture strains, isolated by ourselves from fetuses, and fetal membranes, grown on glycerin-bile-serum agar, were used. Some of these strains were extremely virulent to rats, where others proved to be extremely low in virulence, and consequently also of low toxicity. This fact was determined only after some "abortin" prepared with such strains had been used for tests. The general technic in preparing the "abortin" was as follows:

So-called Holth flasks,⁷ containing glycerin broth of the same reaction (+1.2) as used for the preparation of tuberculin in the laboratory, were inoculated with *B. abortus*. The flask, properly saturated with oxygen, as explained by Holth, was kept

¹ *Ztschr. f. Infektionskr., d. Haustiere*, 1911, 10, p. 342.

² *Deutsche tierärztl. Wchnschr.*, 1911, 19, p. 785.

³ *Berliner tierärztl. Wchnschr.*, 1911, 27, p. 721.

⁴ *Veterinary News*, 1910, 7, p. 697.

⁵ *Annual Report*, Bureau of Animal Industry, 1911, p. 175.

⁶ *Proceedings of the A.V.M.A.*, 1912, p. 345.

⁷ *Ztschr. f. Infektionskr., d. Haustiere*, 1911, 10, p. 217.

at 37° C. for from 6 to 16 weeks. An addition of grape sugar or serum did not improve the growth or the quality of the preparation. Liver broth gave uniformly better results than beef broth. After the time mentioned above had elapsed, and an extremely heavy flocculent deposit was found in the flasks, the cultures were tested for purity. The supernatant fluid was usually turbid at the beginning of the growth, but cleared up gradually as the culture became older. The pure cultures of different strains were poured together, killed by keeping them for one hour in the board of health sterilizer, and then concentrated if possible by vacuum at 60° to 70° C. to one-tenth of its volume. The slow extraction at a low temperature seems also for "abortin" to be essential for a good preparation. The syrupy fluid was filtered through hardened filter paper, and kept in sterile brown bottles at a temperature of 8° C. For use it was always diluted with a saline solution containing 1 per cent glycerin and 0.5 per cent carbolic acid.

As will be shown by the detailed account of our experiments, we found that a "purified abortin" acts more specifically than the preparations just mentioned. From our observations in the conjunctival test for glanders, we realized that an old preparation, particularly when kept in powder form, is more specific. This is undoubtedly due to the fact that the unspecific pyrogenous substances disintegrate rather quickly. A special diagnostic, the so-called "precipitated dry abortin," was prepared. Its manufacture was in our hands as follows:

Twelve-weeks-old cultures, obtained as above, and concentrated at 40° C. to one-tenth of its volume, were first centrifuged and then filtered. A comparatively clear brownish syrupy fluid was obtained. One part of this liquid was precipitated with 20 parts of absolute alcohol, and washed with absolute alcohol and ether. The precipitate, filtered off, was smeared on sterile clay plates and dried over sulfuric acid in vacuum. A granular whitish powder was finally obtained.

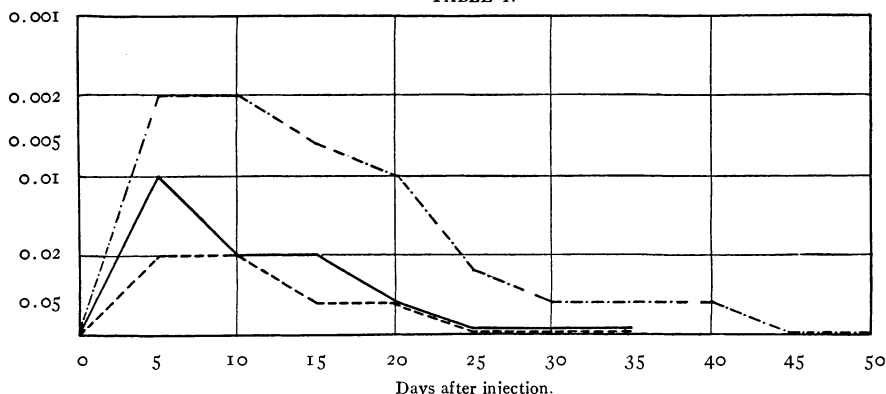
This preparation is not hygroscopic, and is very readily soluble in saline solution. Out of 10 c.c. "abortin br te," that is, the above-named concentrated glycerin broth culture, about 0.95 gm. of powder was received. This preparation kept well in brown bottles, in an exiccator. For the experiments it was dissolved in saline solution.

To test the antigenic properties of our various preparations 3 rabbits for each preparation were inoculated intravenously with 1 c.c. "abortin br te" (10 c.c. diluted or 0.1 gram precipitated "abortin" in 1 c.c. saline). The results are shown graphically in Table 1.

These comparatively few tests show that the highest amount of antigen is found in the "precipitated abortin." The immune

bodies appeared in the rabbits, between the second and the fifth day, remained present for about 10–15 days, but after 45 days had entirely disappeared. Ordinary "abortin brute" from rather young cultures produced only a very small amount of immune bodies; they disappeared in 25 days from the blood of the inoculated animals. "Abortin" from old cultures produced more immune bodies, but these disappeared as quickly as the others. These observations stand in correlation with similar experiments of Zwick and Zeller.

TABLE 1.



Graphic demonstration of the average agglutination titre of 12 rabbits which have been inoculated with the "abortin" preparation used for the tests. One c.c. "abortin brute" (=10 c.c. dilute or filtrate, or 0.1 gram precipitate in 1 c.c. saline) was injected intravenously.

———— = W/4 strain 47-days-old culture.

----- = 2445/3 strain 4-weeks growth.

- · - · - · - = Na 2916 strain 92-days-old culture, precipitated and kept in powder form.

From these preliminary tests we conclude that the "precipitated dry abortin," particularly from old cultures, has greater antigenic properties than the ordinary "abortin brute," a point which is noteworthy when comparing later the practical test results obtained with these products.

In addition to these tests on rabbits, the "abortin" was also used as an antigen in the complement fixation test, with a standard serum of the titre 0.002. Uniformly the antigen titrated to 0.01 c.c., and the "abortin" had a titre of 0.01. In this test we found that no difference exists between the titre of the "ordinary abortin brute" and the "precipitated abortin." The technic of this test,

which undoubtedly permits a standardization of the "abortin" preparations, is extremely simple. Descending doses of "abortin" are tested with complement, hemolysin, and blood. At the same time the same amount of "abortin" is brought in contact with three units of a standard serum, in our particular case 0.006 c.c. serum. Titrated amounts of complement, hemolysins, and blood are added. We can recommend this method based on a large number of tests.

THE TESTS WITH "ABORTIN."

In conjunction with the "abortin" test, naturally serum tests were carried out. The technic for the latter is the same as recommended by Sven Wall¹ and Holth.² Since 1912 these serum tests for infectious abortion have been used in our laboratory as the practical routine tests. They have given uniformly accurate and valuable results.

Before the "abortin" was applied, in every instance blood was collected for the serum tests. For some of the tests we were unable, on account of the large amount of other routine work, to carry out both the agglutination and complement fixation tests simultaneously; as the complement fixation test is more reliable for immunity tests than the agglutination test, it was, therefore, applied as a single test. If in any way possible a retest was made 6 to 12 months after the first test. The interpretation of these tests was the same as recommended and proven by Sven Wall, namely, agglutination ≥ 0.05 and complement fixation ≥ 0.05 is considered a positive reaction. For the agglutination reaction a titre of ≥ 0.01 and for the complement fixation test a reaction with ≥ 0.05 was accepted as positive.

GENERAL TECHNIC OF THE "ABORTIN" TESTS.

In a similar manner as recommended for the tuberculin test two to three preliminary temperatures were taken. The injection of the "abortin" was made subcutaneously or intravenously. Following the injection the temperature readings were made at intervals of two hours. The last temperature was taken usually at the

¹ *Ztschr. f. Infektionskr., d. Haustiere*, 1911, 10, pp. 23 and 132.

Ibid., pp. 206 and 342.

eighteenth or twenty-fourth hour. If, following the "abortin" injection, a rise of temperature was recorded it began between the fourth and twelfth hour, and remained at its height for from 2-16 hours. Occasionally "late" and "atypical" reactions were also recorded. The interpretation of these reactions was made according to the rules adopted by the Eighth International Veterinary Congress in Budapest; namely, an increase of temperature above 40°C . indicates a reaction, if, at the time of the "abortin" injection, the temperature is not over 39.5°C . For the recording of the temperatures "Reform" thermometers (celsius) or "Fahrenheit" thermometers (often two or three at a time) were used.

Local reactions were never noticed as long as only quantities of 1 c.c. "abortin br te" were inoculated. In some instances 5 or 10 c.c. of this preparation were applied. A large, hard, and painful swelling developed which disappeared very slowly. Often 2 to 3 weeks elapsed before it was entirely absorbed. Never was any abscess formation noticed.

Some of the animals, following the intravenous injection, showed general symptoms, trembling, slobbering, accelerated respiration, inappetency, and a reduced secretion of milk. The number of these occurrences was very small. For further details we refer to the experiments.

At this time we have tested altogether 270 animals naturally infected with *B. abortus*. Also some artificially infected animals were tested. The results of these tests were as follows:

"ABORTIN" TEST ON ARTIFICIALLY INFECTED ANIMALS.

For the test 3 heifers, Nos. 45, 46, and 47, stood at disposal. The serum tests showed that the animals had a high agglutination and complement fixation titre. The application of the "abortin" was made subcutaneously. The result of this test is shown in Table 2.

In considering the rules mentioned, Heifers 45 and 46 gave a positive reaction to the subcutaneous "abortin" injection. Heifer 47, which was fed with vaginal discharge, failed to give a reaction. This fact is rather astonishing inasmuch as an agglutination reaction of 0.002 and a complement fixation of 0.02 were recorded.

These experiments show that the "abortin" is not an absolutely reliable diagnostic for artificially infected animals.

"ABORTIN" TEST ON NATURALLY INFECTED ANIMALS.

Herd No. 1.—The dairy herd of the Experimental Farm, numbering 49 animals, in which infectious abortion has existed for the past five years, was used for this test.

TABLE 2.

ANIMAL NUMBER	MODE OF INJECTION	SERUM TESTS AUG. 2, 1912		TEMPERATURES BEFORE INJECTION AUG. 2, 1912			TEMPERATURES AFTER SUBCUTANEOUS INJECTION OF 10 C.C. OF ABORTIN AT THE NECK AT 8.00 A.M.								RESULT OF ABORTIN TEST	HISTORY
		Agglu- tination	Comple- ment Fixation	5:00 A.M.	6:00 A.M.	7:00 A.M.	10:00 A.M.	12:00 M.	2:00 P.M.	4:00 P.M.	6:00 P.M.	8:00 P.M.	10:00 P.M.	12:00 P.M.		
45.....	100 c.c. broth, cul- ture, 6/1/12, intra- venously.....	>0.001	=0.05	100.2	100.6	100.4	102.3	101.6	102.4	103.2	103.6	104.4	104.4	102.1	102.4	Premature calf, 11/8/ 12. <i>B. abortus</i> isolated from fetal membranes.
46.....	100 c.c. broth, cul- ture, 6/1/12, intra- venously.....	>0.001	=0.05	102.2	102.	101.8	101.8	103.1	104.	104.3	103.8	102.4	102.5	102.9	102.2	Calved normally, 12/ 31/12. <i>B. abortus</i> isolated from cotyle- dons.
47.....	Fed vaginal discharge of several aborters for ten days; 5/1/12	=0.002	=0.02	101.3	101.2	101.4	101.2	101.4	101.5	102.2	102.8	102.	101.8	102.2	101.7	Was bred in May, 1913, for the first time with success. Aborted probably very small fetus.

The "abortin" applied was a mixture of 4 different cultures, was not concentrated, but simply passed through Berkefeld candles and preserved with 0.5 per cent carbolic acid. The injection was made subcutaneously. The results of the test are shown in Table 3.

At the time of the test 23 animals (Nos. 1, 2, 6, 7, 9, 10, 12, 14, 16, 17, 19, 20, 21, 29, 31, 34, 41, 42, 45, 46, 47, 48, 49) reacted to the serum test. Out of these reactors 10 had aborted (Nos. 6, 7, 9, 16, 17, 19, 45, 46, 48, 49). By means of the "abortin" test 13 animals (Nos. 4, 13, 14, 20, 25, 26, 27, 29, 31, 32, 33, 46, 48) were found to react to the inoculation, with fever over 104° F. and with a curve which is characteristic for a positive tuberculin reaction. The differences in temperature ranged from 1.6° to 5.2° F. Out of these 13 animals six (Nos. 14, 20, 29, 31, 46, 48) were reactors to the serum test, whereas seven (Nos. 4, 13, 25, 26, 27, 32, 33), which also reacted to the inoculation, were, according to the serum tests, not infected with *B. abortus*. Several animals (Nos. 16, 17, 45, and 49), which had aborted during the last year, and of which Nos. 16, 45, and 49 gave positive reactions to the serum tests, failed to react to the "abortin." No local reactions nor general symptoms were noticed.

Conclusions.—The test applied to Herd No. 1 showed that the inoculation of "abortin" is a very unreliable method of diagnosing the existence of the disease in a herd, particularly when broth filtrates are applied subcutaneously. The results show that a particularly high percentage (53.8 per cent) of the entire "abortin" reactions are found in the non-infected, healthy animals. The test, therefore, is considered extremely unreliable and unspecific with this preparation. An interesting feature has to be mentioned here, namely, several of the animals, which had reacted to the "abortin," but were not affected with *B. abortus*, according to the serum tests, had been subjects of various tests with tuberculin preparations, or had been vaccinated with large doses of human tubercle bacilli according to the method of Pearson. Several of the animals were also badly affected with tuberculosis as subsequent detailed autopsies revealed. We are not in a position to explain satisfactorily this occurrence of reaction in such animals, but suspect that they are probably due to proteins or split products present in the broth.

Herd No. 2.—A dairy herd, consisting of 19 animals, in which recently 2 cases (Nos. 6 and 8) of infectious abortion had been bacteriologically determined, was tested with "abortin." The preparation used was a mixture of cultures similar to that used in Herd No. 1, but concentrated to one-tenth of its volume, and the "abortin brute" dissolved in the quantity of 1-9 c.c. dilution fluid, as mentioned in the introduction. Each 10 c.c. contained, therefore, 1 c.c. of "abortin brute." Injections were made subcutaneously. The results obtained are shown in Table 4.

According to the serum tests three animals (Nos. 6, 8, and 16) were infected. Out of these three, two (Nos. 6 and 8) had aborted recently. Both animals which had aborted reacted also to the "abortin" test, but in addition to that, two more animals (Nos. 2 and 19) which were not affected, according to the serum tests, reacted to the "abortin" injection.

Conclusions.—The results with concentrated and rediluted "abortin" by subcutaneous injection are accurate, particularly when recent aborters are tested; still, inasmuch as two reactions occurred in healthy, non-infected animals, the reactions did not seem to be specific.

In this herd granular vaginitis existed at the same time to a large extent, and by the test applied, the observation and deduction could be made that this local disease

TABLE 3.
HERD NO. 1.

ANIMAL NUMBER	SERUM TEST	TEMPERATURES BEFORE INJECTION AUG. 2, 1912			TEMPERATURES AFTER SUBCUTANEOUS INJECTION OF 10 C.C. OF ABORTIN AT 8:00 A.M. AUG. 2 AND 3, 1912								RESULT OF ABORTIN TEST	HISTORY		
		INTER- PRETA- TION OF SERUM TEST	TEMPERATURES BEFORE INJECTION AUG. 2, 1912		10:00 A.M.	12:00 M.	2:00 P.M.	4:00 P.M.	6:00 P.M.	8:00 P.M.	10:00 P.M.	12:00 P.M.			2:00 A.M.	
			5:00 A.M.	6:00 A.M.												7:00 A.M.
1.....	=0.05	Positive	101.4	101.	101.9	101.1	101.6	102.1	101.9	102.1	101.7	102.4	101.8	Premature calf. Never aborted.	
2.....	=0.02	"	101.0	101.2	101.6	101.4	101.8	101.9	102.5	102.3	102.3	102.4	101.6		
3.....	=0	101.6	101.5	102.2	100.8	102.	103.	102.6	103.	102.2	102.2	101.7		
4.....	=0.1	101.7	101.3	102.6	101.5	102.	103.8	104.3	104.3	103.8	104.3	104.4	Positive		
5.....	=0	Positive	101.4	101.6	102.	101.	101.3	102.3	102.6	102.9	102.4	102.4	102.	"	" 12/28/07. " 1/19/10. " 2/13/10. " 5/15/07. " 7/ 6/09.	
6.....	=0.02		101.1	101.1	101.4	101.2	101.6	102.3	102.4	102.6	102.6	102.1	101.2	102.2		
7.....	=0.05	"	101.3	101.5	102.	101.5	102.	103.	102.6	102.6	102.6	102.1	102.7		
8.....	=0	101.1	101.8	102.1	100.7	101.1	102.8	103.	103.	102.8	102.7	102.7		
9.....	=0.02	Positive	102.	102.2	102.	101.1	101.6	102.7	103.4	103.7	103.2	102.8	103.2	
10.....	>0.005	"	101.1	101.2	101.8	101.1	101.3	102.4	102.6	102.3	101.8	102.	101.8		
11.....	=0	Positive	101.	101.1	101.6	101.1	101.1	102.1	102.6	102.6	102.8	101.	102.6		
12.....	>0.005	"	101.5	101.	102.3	102.8	102.	103.	103.5	103.1	102.7	101.8	101.4	Positive		
13.....	=0.02	Positive	101.3	102.	102.	101.8	102.2	102.	104.	105.2	105.2	102.2	103.6	Difficult to breed.	
14.....	=0	"	101.2	101.6	101.6	101.6	103.	103.7	102.3	103.1	102.5	104.7	102.6			
15.....	=0.05	Positive	101.1	101.5	102.4	101.3	101.1	102.8	102.3	103.2	102.6	103.	102.1			Aborted, 5/2/11. " 6/10/11.
16.....	=0.05	"	101.3	101.3	102.6	101.1	101.9	102.8	103.4	103.	102.9	102.4	102.1			
17.....	=0.05	101.4	101.2	101.4	101.4	102.2	102.8	102.7	103.	103.1	102.2	102.8	Aborted, 12/28/07.		
18.....	=0.05	Positive	101.2	101.3	101.6	100.6	100.9	102.3	101.9	102.7	102.1	102.5	102.8		Positive	
19.....	=0.02	"	102.4	102.6	102.3	102.	104.1	104.2	105.2	105.	104.5	101.6	102.7		
20.....	=0.02	101.2	101.4	101.8	100.7	101.2	102.6	102.3	103.	102.4	103.	102.3			
21.....	=0.1	"	101.3	101.6	101.9	100.7	101.2	102.3	102.7	103.5	102.1	102.9	101.8	Positive		
22.....	=0	"	101.4	101.6	101.5	100.7	101.2	102.3	102.7	103.1	102.2	101.9	102.2		Positive	
23.....	=0	"	101.8	102.2	102.	100.4	101.2	102.4	102.8	103.1	102.2	101.9	102.2			Positive
24.....	=0.01	"	102.3	101.9	102.2	100.9	102.	103.1	104.8	104.6	102.3	105.1	102.2			
25.....	=0	102.	102.3	102.2	102.	102.5	103.1	104.3	104.8	105.8	105.	104.8	Positive		
26.....	=0	"	102.	102.	102.9	101.5	102.3	104.3	106.4	107.2	105.8	105.	101.8		Positive	
27.....	=0	101.9	102.1	101.8	100.3	101.4	103.5	102.8	103.4	102.6	102.5	102.			Positive
28.....	=0	"	102.	101.8	102.4	100.3	102.6	105.2	104.9	104.3	103.4	103.6	102.8			
29.....	=0.02	Positive	101.5	101.8	102.	102.3	102.6	105.2	102.5	103.6	103.4	103.6	103.4	Positive		
30.....	=0	"	101.5	102.	103.	101.6	101.6	101.7	102.5	103.6	106.3	103.1	105.4		Positive	
31.....	=0.05	Positive	101.9	102.6	102.4	101.9	102.3	103.9	105.5	106.6	106.3	103.1	105.4			" 105.8
32.....	=0	"	101.6	102.2	102.4	101.7	101.8	104.1	103.7	106.	105.	103.7	104.3			
33.....	=0	"	102.8	103.	103.6	102.5	102.5	102.5	104.9	103.7	102.6	103.3	103.2	Difficult to breed.		
34.....	=0.02	Positive	101.8	101.7	101.8	101.7	102.	102.9	102.8	102.4	102.6	103.3	102.		

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*Animals 33-40 inclusive and animals 43-40 inclusive received 5 c.c. subcutaneously. Animals 40, 41, and 42 were inoculated intravenously with 10 c.c.

TABLE 4.
HERD No. 2.

ANIMAL NUMBER	SERUM TEST	INTERPRE- TATION OF SERUM TESTS	TEMPERATURES BEFORE INJECTION AUG. 20, 1912				TEMPERATURES AFTER SUBCUTANEOUS INJECTION OF 10 C.C. OF ABORTIN AT 2.15 P.M. AUG. 20 AND 21, 1912								RESULT OF THE ABORTIN TEST	HISTORY	
			TEMPERATURES BEFORE INJECTION AUG. 20, 1912				TEMPERATURES AFTER SUBCUTANEOUS INJECTION OF 10 C.C. OF ABORTIN AT 2.15 P.M. AUG. 20 AND 21, 1912										
			9:00 A.M.	11:30 A.M.	12:30 P.M.	2:00 P.M.	4:00 P.M.	6:00 P.M.	8:00 P.M.	10:00 P.M.	12:00 P.M.	2:00 A.M.	4:00 A.M.	6:00 A.M.			8:00 A.M.
1	= 0	101.9	101.8	101.5	102.5	102.6	101.8	102.4	101.8	101.7	100.6	102.2	100.	100.7	Granular Vaginitis. " " " " " "
2	= 0	102.9	102.5	102.5	103.8	102.6	101.9	103.3	103.6	102.9	102.4	101.9	101.9	101.4	
3	= 0	101.8	101.1	101.6	101.8	101.7	102.1	102.8	102.6	102.2	101.5	101.	101.7		
4	= 0	102.3	101.8	102.1	102.4	101.8	101.8	102.9	102.8	101.9	101.2	100.6	100.4	100.7	
5	= 0	103.3	103.2	103.9	104.4	103.6	103.9	104.4	103.7	103.6	103.	103.	101.9	102.5	Aborted, May, 1912; Granular Vaginitis.
6	= 0.05	Positive	103.6	103.5	103.6	104.4	103.2	100.3	104.6	105.2	104.4	104.3	103.6	102.4	102.2	Positive	
7	= 0	102.1	101.8	101.8	102.9	102.2	103.3	103.9	103.	103.2	102.5	102.9	100.	100.8	Granular Vaginitis. Aborted, July, 1912; Granular Vaginitis.
8	= 0.05	Positive	102.6	102.1	101.8	102.7	101.8	101.5	103.3	103.1	103.	103.4	104.6	103.2	103.1	Positive	
9	= 0	102.1	100.7	101.5	101.8	100.3	100.4	101.7	100.6	100.9	100.	101.	100.7	100.7	Granular Vaginitis. " " " " " "
10	= 0	103.8	103.7	104.	104.3	103.2	102.8	104.	104.	103.3	103.	103.6	101.1	101.1	
11	= 0	102.2	101.5	102.2	103.	102.	102.9	103.5	103.6	103.	102.9	103.	102.5	102.4	
12	= 0	102.4	102.4	103.	103.9	103.6	103.	104.	103.6	102.9	102.3	101.8	101.8	101.8	Positive	
13	= 0	102.7	101.9	102.3	103.	102.	101.7	102.8	102.9	102.	101.7	101.8	102.	102.	" "
14	= 0	103.	102.7	103.4	104.2	101.4	102.5	104.	104.2	103.5	103.2	102.1	101.2	101.2	
15	= 0	102.5	102.1	102.4	102.9	101.	101.4	102.4	103.7	102.4	101.4	101.4	101.1	101.2	" "
16	= 0.02	Positive	102.4	102.	102.5	103.5	101.9	101.9	101.9	101.9	101.9	101.8	101.6	101.	101.	
17	= 0	102.5	102.3	102.7	103.	102.	102.2	103.	102.6	102.3	101.7	101.3	101.4	101.3	" "
18	= 0	102.6	102.1	102.7	103.	101.	101.6	101.9	102.	101.4	100.4	100.5	100.9	100.4	
19	= 0	102.8	102.	102.6	102.7	102.	102.7	102.3	102.6	103.	103.	104.	103.6	103.6	Positive	" "

TABLE 5.
HERD No. 4.

ANIMAL NUMBER	AGE	SERUM TESTS				INTER- PRETA- TION OF SERUM TESTS	TEMPERA- TURES BEFORE INJECTION DEC. 7, 1912		INJECTION DOSE OF ABORTIN AT 1:00 P.M.	TEMPERATURES AFTER INJECTION DEC. 7 AND 8, 1912							
		1st Agglu- tination 10/30/12	1st Comple- ment Fixation	2d Agglu- tination 12/7/12	2d Comple- ment Fixation		7:00 P.M.	11:00 A.M.		4:00 P.M.	6:00 P.M.	8:00 P.M.	10:00 P.M.	12:00 P.M.	2:00 A.M.	4:00 A.M.	6:00 A.M.
1.....	9	>0.1	=0.005	>0.01	=0.005	Positive	38.5	38.2	10 c.c. subcutaneously	38.6	38.2	38.2	38.4	38.1	38.6	38.2	38.3
2.....	10	=0.02	=0.02	=0.05	=0.05	"	38.6	38.0	10 " intravenously	37.9	38.5	38.5	38.0	38.2	38.6	39.8	38.3
3.....	9	=0.02	=0.005	>0.01	=0.005	"	38.1	38.5	2.5 " intravenously	38.4	38.2	38.4	38.1	38.5	38.7	38.6	38.3
4.....	9	=0.05	=0	=0.05	=0	39.0	38.9	10 " subcutaneously	39.2	38.7	39.1	39.1	39.1	38.6	38.5	38.3
5.....	4	=0.02	=0.05	=0.02	=0.02	Positive	38.4	38.2	2.5 " intravenously	38.9	41.4	41.5	40.4	39.0	39.0	39.8	38.3
6.....	4	0	=0	0	=0	38.4	38.4	10 " subcutaneously	38.3	38.4	38.4	38.2	38.3	38.1	38.2	38.3
7.....	7	=0.05	=0	0	=0	38.3	38.1	10 " " "	38.1	38.3	38.3	38.3	38.1	38.2	38.5	38.3
8.....	6	>0.01	=0.05	>0.01	=0.005	Positive	38.3	38.1	5 " " "	38.3	38.5	38.5	38.4	38.4	38.6	39.1	38.3
9.....	11	=0.05	=0	0	=0	38.0	38.2	2.5 " intravenously	38.0	38.1	38.4	38.4	37.9	37.9	38.4	38.3
10.....	16	=0.05	=0.02	>0.01	=0.05	Positive	38.2	38.5	5 " subcutaneously	38.7	38.5	39.1	38.7	38.6	38.2	38.3	38.3
11.....	11	=0.02	=0	=0.05	=0.1	38.4	38.1	2.5 " intravenously	38.2	38.1	38.5	38.2	38.1	38.0	38.3	38.3
12.....	13	>0.01	=0.02	>0.01	=0.005	Positive	38.9	38.2	10 " subcutaneously	38.3	38.6	38.5	38.6	39.4	40.1	40.5	40.4
13.....	8	>0.01	=0.005	>0.01	=0.05	"	38.7	38.3	10 " " "	38.6	38.6	38.8	39.1	39.0	39.1	39.3	38.3
14.....	4	0	=0	0	=0	38.6	37.9	10 " " "	38.1	38.4	38.6	38.0	38.0	38.0	38.2	38.3
15.....	5	>0.01	=0.02	=0.02	=0.05	Positive	39.1	38.4	2.5 " intravenously	39.0	38.7	39.3	39.1	38.9	38.7	38.6	38.3
16.....	2	=0.02	=0.05	>0.01	=0.02	"	38.3	38.1	2.5 " " "	38.5	38.8	39.4	39.4	39.4	39.0	39.0	38.3
17.....	4	=0.02	=0.05	>0.01	=0.005	"	39.1	38.4	2.5 " " "	38.4	39.1	40.4	41.1	41.2	40.8	40.5	38.3
18.....	2	=0.02	=0.05	=0.02	=0.05	"	38.3	38.3	2.5 " " "	38.2	38.6	40.2	40.7	40.3	39.8	39.4	38.3
19.....	2	>0.01	=0.05	>0.01	=0.1	"	38.5	38.1	2.5 " " "	38.2	38.0	38.6	38.5	38.4	38.7	38.6	38.3
20.....	3	=0.05	=0.05	=0.05	=0	38.6	38.5	2.5 " " "	38.0	38.1	38.0	38.7	37.9	38.3	38.4	38.3
21.....	5	=0.02	>0.005	>0.01	=0.005	Positive	38.4	38.6	10 " subcutaneously	39.0	38.0	38.6	39.1	39.7	40.0	39.5	38.3
22.....	5	=0.05	=0	0	=0	38.7	38.2	2.5 " intravenously	38.2	37.9	38.1	38.5	38.1	38.2	38.2	38.3
23.....	7	=0.05	=0	0	=0	38.2	38.4	2.5 " " "	38.8	38.4	38.3	38.5	38.6	38.2	38.6	38.3
24.....	3	>0.01	=0.05	=0.02	=0.05	Positive	38.3	38.5	2.5 " " "	39.1	38.7	39.4	38.7	40.0	40.6	40.6	38.3
25.....	13	=0.05	=0	0	=0	38.9	38.4	2.5 " " "	38.8	38.7	39.1	39.0	38.9	38.8	38.6	38.3
26.....	13	=0.05	=0	=0.05	=0	38.8	38.4	2.5 " " "	39.0	38.8	38.8	38.8	38.6	38.9	38.8	38.3
27.....	13	=0.05	=0	0	=0	38.6	38.3	2.5 " " "	38.7	38.4	38.3	38.4	38.2	38.6	38.9	38.3
28.....	20	=0.05	=0	=0.05	=0	38.5	38.2	2.5 " " "	38.8	38.3	38.6	38.5	38.2	38.3	38.5	38.3
29.....	20	=0.05	=0	=0.05	=0	39.2	38.4	2.5 " " "	39.0	39.0	38.8	38.9	38.8	39.0	38.6	38.3
30.....	20	=0.05	=0	0	=0	38.5	38.5	2.5 " " "	38.6	38.6	39.4	40.1	39.7	39.6	39.7	38.3
31.....	12	=0.05	=0	0	=0	38.7	38.3	2.5 " " "	38.6	38.8	38.6	38.6	38.7	38.4	38.5	38.3
32.....	10	0	=0.02	0	=0.1	39.7	38.6	10 " subcutaneously	38.5	38.1	38.6	38.9	38.6	38.2	38.2	38.3

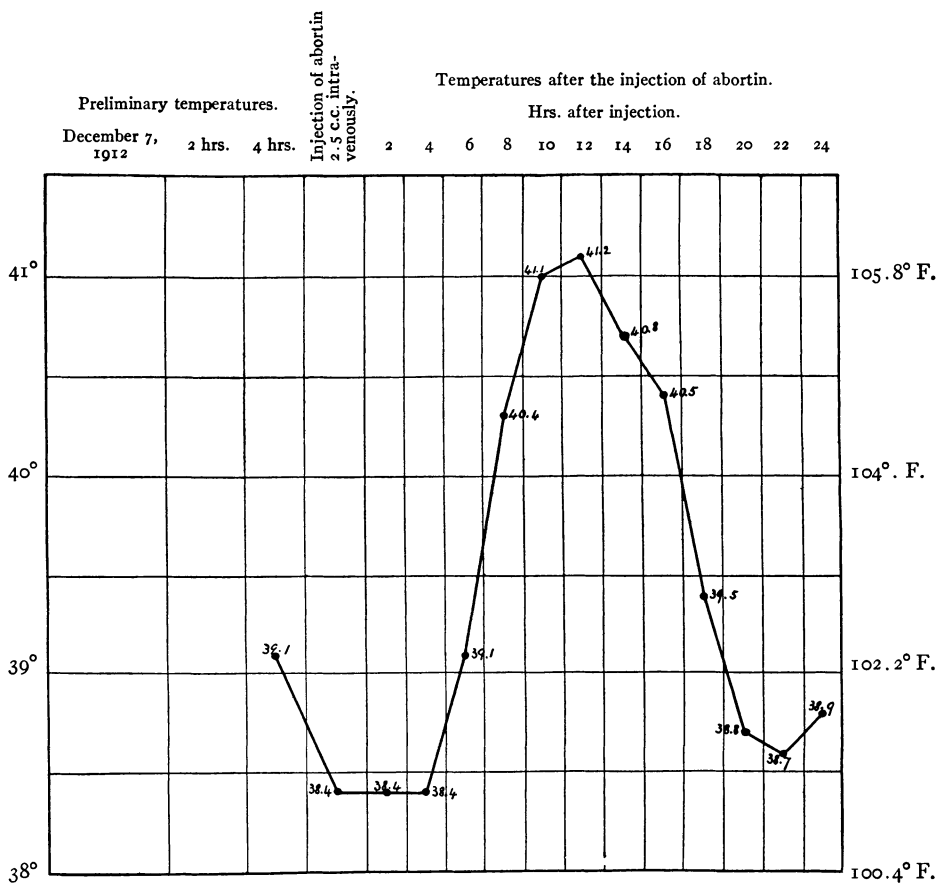
TABLE 5.
HERD No. 4.

INTER- PRETA- TION OF SERUM TESTS	TEMPERA- TURES BEFORE INJECTION DEC. 7, 1912		INJECTION DOSE OF ABORTIN AT 1:00 P.M.	TEMPERATURES AFTER INJECTION DEC. 7 AND 8, 1912										RESULT OF THE ABORTIN TEST	HISTORY
	7:00 P.M.	11:00 A.M.		4:00 P.M.	6:00 P.M.	8:00 P.M.	10:00 P.M.	12:00 P.M.	2:00 A.M.	4:00 A.M.	6:00 A.M.	8:00 A.M.			
Positive	38.5	38.2	10	c.c. subcutaneously	38.6	38.2	38.2	38.4	38.1	38.6	38.2	38.4	38.5	1 aborted previous to 1911.
"	38.6	38.0	10	"	37.9	38.5	38.5	38.0	38.2	38.6	39.8	39.3	39.0	1 aborted, 1/3/12.
"	38.1	38.5	2.5	" intravenously	38.4	38.2	38.4	38.1	38.5	38.7	38.6	38.7	38.4	Never aborted; O.K.; calved nor- mally 12/4/11.
.....	39.0	38.9	10	" subcutaneously	39.2	38.7	39.1	39.1	39.1	38.6	38.5	38.5	38.8	Never aborted.
Positive	38.4	38.2	2.5	" intravenously	38.9	41.4	41.5	40.4	39.0	39.0	39.8	39.4	39.0	1 aborted, 10/17/12.
.....	38.4	38.4	10	" subcutaneously	38.3	38.4	38.4	38.2	38.3	38.1	38.2	38.6	38.4	Never aborted.
.....	38.3	38.1	10	"	38.1	38.3	38.3	38.3	38.1	38.2	38.5	38.7	38.7	"
Positive	38.3	38.1	5	"	38.3	38.5	38.5	38.4	38.4	38.6	39.1	39.0	39.1	Aborted, 8/13/09; calved, 2/11.
.....	38.0	38.2	2.5	" intravenously	38.0	38.1	38.4	38.4	37.9	37.9	38.4	38.4	38.3	Never aborted.
Positive	38.2	38.5	5	" subcutaneously	38.7	38.5	39.1	38.7	38.6	38.2	38.3	38.9	38.9	"
.....	38.4	38.1	2.5	" intravenously	38.2	38.1	38.5	38.2	38.1	38.0	38.3	38.5	38.3	"
Positive	38.9	38.2	10	" subcutaneously	38.3	38.6	38.5	38.6	39.4	40.1	40.5	40.1	39.8	Positive
"	38.7	38.3	10	"	38.6	38.6	38.8	39.1	39.0	39.1	39.3	39.4	39.5	1 aborted, 7/26/11.
.....	38.6	37.9	10	"	38.1	38.4	38.6	38.0	38.0	38.0	38.2	38.7	38.6	2 abortions, 1908, 5/11/11.
Positive	39.1	38.4	2.5	" intravenously	39.0	38.7	39.3	39.1	38.9	38.7	38.6	38.8	38.8	Never aborted.
"	38.3	38.1	2.5	"	38.5	38.8	39.4	39.4	39.4	39.0	39.0	39.4	39.4	"
"	39.1	38.4	2.5	"	38.4	39.1	40.4	41.1	41.2	40.8	40.5	39.3	38.8	1 abortion, 9/3/12.
"	38.3	38.3	2.5	"	38.2	38.6	40.2	40.7	40.3	39.8	39.4	39.3	40.8	2 abortions, 7/20/10, 4/27/11.
"	38.5	38.1	2.5	"	38.2	38.0	38.6	38.5	38.4	38.7	38.6	39.0	39.1	1 abortion, 9/22/12.
.....	38.6	38.5	2.5	"	38.9	38.1	38.0	38.7	37.9	38.3	38.4	38.3	38.7	1 abortion, 10/12/12.
Positive	38.4	38.6	10	" subcutaneously	39.0	38.0	38.6	39.1	39.7	40.0	39.5	38.5	38.3	Calved, 3/28/12 (never aborted).
.....	38.7	38.2	2.5	" intravenously	38.2	37.9	38.1	38.5	38.1	38.2	38.2	38.1	38.7	Aborted, 4/10/10.
Positive	38.2	38.4	2.5	"	38.8	38.4	38.3	38.5	38.6	38.2	38.6	38.4	38.4	Never aborted.
.....	38.3	38.5	2.5	"	39.1	38.7	39.4	38.7	40.0	40.6	40.6	39.5	39.3	"
.....	38.9	38.4	2.5	"	38.8	38.7	39.1	39.0	38.9	38.8	38.6	38.7	39.0	"
.....	38.8	38.4	2.5	"	39.0	38.8	38.8	38.8	38.6	38.9	38.8	38.8	39.1	Never aborted; not yet calved.
.....	38.6	38.3	2.5	"	38.7	38.4	38.3	38.4	38.2	38.6	38.9	39.2	39.3	" " " " "
.....	38.5	38.2	2.5	"	38.8	38.3	38.6	38.5	38.2	38.3	38.5	38.6	38.8	" " " " "
.....	39.2	38.4	2.5	"	39.0	39.0	38.8	38.9	38.8	39.0	38.6	38.8	38.9	" " " " "
.....	38.5	38.5	2.5	"	38.6	38.6	39.4	40.1	39.7	39.6	39.7	39.6	40.2	Positive
.....	38.7	38.3	2.5	"	38.6	38.8	38.6	38.6	38.7	38.4	38.5	39.1	38.4	" " " " "
.....	39.7	38.6	10	" subcutaneously	38.5	38.1	38.6	38.9	38.6	38.2	38.2	38.5	39.1	1 abortion, 9/12/10.

of the genital tract is not responsible for abortion. This is only a confirmation of what has been reported by Zwick and Zeller, Eber and others.

Herd No. 4.—In a herd of 32 pure-bred Guernseys, in which infectious abortion had existed for the last four years, we were able to apply the "abortin" test by somewhat different technic. The preparation used was a mixture of 8-weeks-old cultures concentrated and rediluted as previously mentioned. The application was made subcutaneously and intravenously. The results of the test are shown in Table 5.

TABLE 5a.



Cow 17, Herd 4. Aborted twice July 20, 1910 and April 27, 1911.

Abortin test December 7, 1912; Serum tests December 7, 1912.

Agglutination: <0.01.

Complement fixation: 0.005.

Highest temperature before injection 102.4° F.

Highest temperature after injection 106.1° F.

Difference in temperature $\frac{106.1}{3.7}$ ° F.

Reaction: positive.

According to the serum tests 15 animals (Nos. 1, 2, 3, 5, 8, 10, 12, 13, 15, 16, 17, 18, 19, 21, 24) were affected with *B. abortus*. Altogether 12 animals (Nos. 1, 2, 5, 8, 12, 13, 16, 17, 18, 19, 21, 32) had aborted in this herd. Out of these 12 animals seven (Nos. 5, 12, 17, 18, 21, 24, 30) reacted to the "abortin." The temperature rise varied between 1.5° and 3.1° C.

In Table 5a, a positive reaction in curve form illustrates the reaction. Out of the healthy ones only one animal (No. 30) reacted atypically, this animal being a heifer.

In Table 6, below, the results obtained by the intravenous and subcutaneous methods are shown in comparison:

TABLE 6.

INTRAVENOUSLY: 20				SUBCUTANEOUSLY: 12			
Aborters	Aborted in Last Two Years	Reactors to Serum Tests	Reactors to Abortin	Aborters	Aborted in Last Two Years	Reactors to Serum Tests	Reactors to Abortin
5	5	8	5 (62.5 per cent)	7	7	7	2 (28.5 per cent)
Healthy: 1				Healthy: none			

In comparing the results obtained by the different methods, we find, as shown in Table 6, that 62.5 per cent were diagnosed when the "abortin" was applied intravenously. Only 28.5 per cent were found to react to the diagnostic following the subcutaneous application. Out of the healthy animals one animal reacted to the intravenous method, and none to the subcutaneous method.

Conclusions.—From the "abortin" tests on 32 animals inoculated by different methods (subcutaneous and intravenous) with old concentrated cultures, we conclude that the intravenous method gives somewhat more accurate results than the subcutaneous method. Still the prevailing non-specificity of the reaction in the other tests is not eliminated by the intravenous method.

Herd No. 5.—The conclusions drawn from the experiments on Herd No. 4 warranted some further tests. An opportunity was offered to select 22 animals out of a herd of 138 pure-bred animals. These animals were tested by the intravenous and subcutaneous methods with an old concentrated, rediluted "abortin" of known antigenous properties. The results obtained are shown in Table 7.

Out of the 14 reactors (Nos. 18, 127, 128, 129, 130, 131, 72, 10, 42, 134, 135, 136, 137, 138) to the serum tests, 12 animals (Nos. 127, 128, 129, 130, 131, 72, 42, 134, 135, 136, 137, 138) had recently aborted. Thirteen animals (Nos. 7, 18, 31, 32, 127, 128, 129, 130, 131, 72, 42, 133, 138) altogether reacted to the "abortin," with a temperature rise which varied between 1.9° and 3.2° C. Two animals, following the intravenous test, showed general symptoms. Nine animals (Nos. 18, 127, 128, 129, 130, 131, 72, 42, 138) reacting to the "abortin" were also reactors to the serum tests. On the other hand, four animals (Nos. 7, 31, 32, 133) reacting to the "abortin" were, according to the serum tests, not infected with *B. abortus*. Neither had any of these animals ever aborted. Animals No. 130 and 131 gave at the first test a high agglutination

TABLE 7.
HERD No. 5.

ANIMAL NUMBER	SERUM TESTS		INTER- PRETA- TION OF SERUM TESTS	TEMPERATURES BEFORE INJECTION MARCH 19, 1913			DATE 3/19/13	TEMPERATURES AFTER INJECTION MARCH 20, 1913							
	1st Agglu- tination 3/1/13	1st Comple- ment Fixation		5.00 P.M.	7:00 P.M.	9:00 P.M.		1:00 A.M.	3:00 A.M.	5:00 A.M.	7:00 A.M.	9:00 A.M.	11:00 A.M.	1:00 P.M.	3:00 P.M.
7.....	0	=0	38.2	37.9	2.5 c.c. intravenously	38.4	38.1	38.4	38.5	38.7	39.1	39.9½	40.
18.....	>0.01	=0.02	Positive	38.7	38.5	" "	38.8	38.7	38.5	38.7	38.8	39.6	40.1½	40.3
31.....	>0.01	=0	38.6	38.3	" "	38.7	38.9	40.8	40.5	40.8	41.3½	39.4	39.8
32.....	0	=0	38.5	38.5	" "	38.5	38.4	38.5	38.5	38.4	38.6½	40.1	40.4
33.....	0	=0	39.2	39.7
127.....	>0.01	=0.02	Positive	38.7	38.5	2.5 c.c. intravenously	38.4	38.	38.4	39.4	41.9½	40.2	40.4	40.1
128.....	>0.01	>0.005	"	38.3	38.3	" "	38.7	38.3	38.5	38.3	38.3	38.4	38.9	39.6
129.....	>0.01	=0.02	"	39.3	39.	38.7	" "	38.8	38.7	39.8	41.3	41.	40.8	40.6	41.3
130.....	>0.01	>0.02	"	38.5	38.	" "	38.4	39.1	40.6	40.2	39.3	38.8	40.4	39.9
131.....	>0.01	>0.005	"	38.6	38.	" "	38.1	38.5	38.5	40.1	41.3	41.1
132.....	>0.01	=0.1	38.2	37.6	" "	37.9	38.2	37.7	38.4	38.4	38.1	38.1	38.4
71.....	0	=0.05	38.2	38.3	" "	38.2	38.5	38.3	38.6	38.1	38.6	39.6	39.9
7e.....	=0.05	=0.02	Positive	38.4	38.4	" "	38.2	38.6	38.3	38.1	38.2	38.7	39.6	40.5
9.....	0	=0	38.5	38.1	10 c.c. subcutaneously	38.2	38.3	38.5	38.3	38.5	38.5	38.7	38.5
10.....	>0.01	=0.05	Positive	38.5	38.3	" "	38.3	38.5	38.8	38.7	38.5	38.5	38.9	38.7
34.....	=0.05	38.5	38.7	" "	39.1	39.4	38.8	38.4	38.4	38.5	38.6	38.7
42.....	>0.01	=0.02	Positive	38.5	39.5	" "	38.4	38.5	38.9	39.5	40.5	40.9	40.4	40.5
133.....	>0.01	0	38.4	38.3	" "	38.2	38.2	38.4	40.9	38.2	38.4	39.5	38.5
134.....	>0.01	>0.005	Positive	38.8	38.3	" "	38.3	38.7	38.9	39.1	39.2	39.	39.0	39.3
135.....	>0.01	>0.005	"	38.9	37.9	" "	38.3	38.5	38.6	38.8	39.	38.9	38.9	38.9
136.....	>0.01	>0.005	"	38.6	38.4	" "	38.2	38.3	38.5	38.7	38.1	38.5	38.7	39.1
137.....	>0.01	>0.005	"	38.9	39.	" "	38.7	38.5	39.	38.8	38.2	38.3	38.6	38.1
138.....	>0.01	>0.005	"	38.4	38.1	" "	38.3	38.9	39.2	39.4	39.7	40.4	39.9	40.2

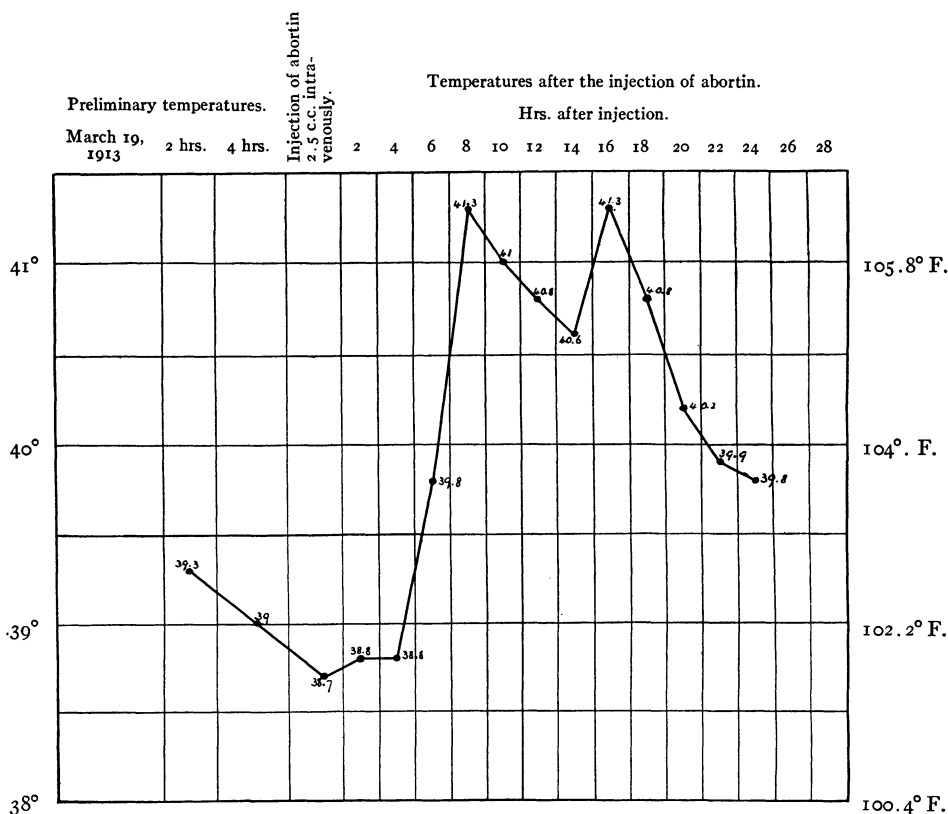
HERD No. 5.

DATE 3/19/13		TEMPERATURES AFTER INJECTION MARCH 20, 1913										RESULT OF THE ABORTIN TEST	HISTORY
Dose of Abortin Injected at 10:00 P.M.		1:00 A.M.	3:00 A.M.	5:00 A.M.	7:00 A.M.	9:00 A.M.	11:00 A.M.	1:00 P.M.	3:00 P.M.	5:00 P.M.	7:00 P.M.		
2.5 c.c. intravenously		38.4	38.1	38.4	38.5	38.7	39.1	39.9½	40.	39.9	40.1	Positive	General symptoms; difficult to breed.
"	"	38.8	38.7	38.5	38.7	38.8	39.6	40.1½	40.3	40.6	40.6	"	Difficult to breed; freshened in Scotland.
"	"	38.7	38.9	40.8	40.5	40.8	41.3½	39.4	39.8	40.8	39.3	"	2d calf bred; calved, 12/8/12.
"	"	38.5	38.4	38.5	38.5	38.4	38.6½	40.1	40.4	39.9	39.	"	Calved normally.
2.5 c.c. intravenously		38.4	38.	38.4	39.4	41.9½	40.2	40.4	40.1	40.9	40.9	Positive	Aborted, 2/4/13; 3d calf.
"	"	38.7	38.3	38.5	38.3	38.3	38.4	38.9	39.6	41.0	41.0	"	1/29/13; 2d calf; bred.
"	"	38.8	38.7	39.8	41.3	41.	40.8	40.6	41.3	40.8	40.2	"	5/21/13. Aborted, 2/4/13; 3d calf; bred.
"	"	38.4	39.1	40.6	40.2	39.3	38.8	40.4	39.9	39.4	39.9	"	5/19/13. Aborted, 2/6/13; 2d calf; bred.
"	"	38.1	38.5	38.5	40.1	41.3	41.1	41.4	41.6	"	5/3/13. Aborted, 12/8/12; trembling; general symptoms.
"	"	37.9	38.2	37.7	38.4	38.4	38.4	38.1	38.4	38.7	38.3	Aborted, 1/17/13.
"	"	38.2	38.5	38.3	38.6	38.1	38.6	39.6	39.9	39.7	38.9	" 1910.
"	"	38.2	38.6	38.3	38.1	38.2	38.7	39.6	40.5	39.6	39.5	Positive	" 1910.
10 c.c. subcutaneously		38.2	38.3	38.5	38.3	38.5	38.5	38.7	38.5	39.1	38.9	Calved normally.
"	"	38.3	38.5	38.8	38.7	38.5	38.5	38.9	38.7	38.3	38.8	3d calf bred.
"	"	39.1	39.4	38.8	38.4	38.4	38.5	38.6	38.7	38.6	38.6	Calved normally, 8/13/12; difficult to breed.
"	"	38.4	38.5	38.9	39.5	40.5	40.9	40.4	40.5	40.2	39.9	Positive	Aborted, June, 1912.
"	"	38.2	38.2	38.4	40.9	38.2	38.4	39.5	38.5	39.3	38.5	"	Calved, 4/15/12.
"	"	38.3	38.7	38.9	39.1	39.2	39.	39.0	39.3	39.6	39.5	Aborted, 2/ 7/13; 5th calf.
"	"	38.3	38.5	38.6	38.8	39.	38.9	38.9	38.9	39.7	39.4	" 2/10/13; 2d "
"	"	38.2	38.3	38.5	38.7	38.1	38.5	38.7	39.1	39.7	39.8	" 2/27/13; 4th "
"	"	38.7	38.5	39.	38.8	38.2	38.3	38.6	38.1	39.1	38.9	" 2/25/13; 1st "
"	"	38.3	38.9	39.2	39.4	39.7	40.4	39.9	40.2	40.7	40.0	Positive	" 3/1/13; 4th calf (prema- ture).

reaction, but no complement fixation. On the retest, which was carried out several weeks afterward, the animals gave a marked complement fixation reaction. Animal No. 133, a heifer just bred, is based on the high agglutination, probably in the stage of incubation, and gave a distinct positive reaction to the "abortin." This is noteworthy for further investigations, with the object of determining if it is not possible that an early infection can perhaps be more readily determined by the "abortin" test.

In comparing again the results of the different methods, intravenous and subcutaneous, we note from Table 8 that by the intravenous method 100 per cent accurate results were obtained; by the subcutaneous method on practically the same number

TABLE 7a.



Cow 129, Herd 5. Aborted February 4, 1913.

Abortin test March 19, 1913. Serum tests March 1, 1913.

Agglutination: <0.01.

Complement fixation: 0.02.

Highest temperature before injection 102.6° F.

Highest temperature after injection 106.2° F.

Difference in temperature $\frac{3.6^\circ \text{F.}}{}$

Reaction: positive.

of reactors and aborters only 18.5 per cent were accurately diagnosed by the "abortin."

On the other hand, the percentage of non-specific reactions by the intravenous method is higher than by the subcutaneous method. The percentage of failures in healthy animals by the intravenous method was 60 per cent and by the subcutaneous, 33.3 per cent.

TABLE 8.

INTRAVENOUSLY: 12				SUBCUTANEOUSLY: 10			
Aborters	Aborted in Last Two Years	Reactors to Serum Tests	Reactors to Abortin	Aborters	Aborted in Last Two Years	Reactors to Serum Tests	Reactors to Abortin
8	6	7	10 (7) (100 per cent)	6	6	7	3 (2) (18.5 per cent)
Healthy: 3 out of 5 animals (60 per cent)				Healthy: 1 out of 3 animals (33.3 per cent)			

Conclusions.—Out of 22 aborters and healthy animals tested by intravenous and subcutaneous "abortin" injections, it was shown that 100 per cent accurate results were obtained by the intravenous method. On the other hand, a rather high percentage of failures was noticed when using this method of injection of the biologic product. The results obtained show again that the "abortin" is non-specific, but that certain valuable information can be obtained by its application, particularly when the intravenous method is used.

Herd No. 6.—To prove the above statements, that the intravenous method may give valuable information, we considered it wise to test a small herd in which abortion had recently occurred, purely by the intravenous method. On April 5, 1913, 18 animals were tested. The "abortin" preparation used for this test was the same as used in Herd No. 5. The gratifying results are shown in Table 9.

According to the serum tests four animals (Nos. 13, 5, 19, 8) were infected with *B. abortus*. One animal (No. 8) had aborted two days previous to the test. All (Nos. 13, 5, 19, 8) the animals which gave positive reactions to the serum tests reacted with a marked elevation of temperature, showing differences between 1.7° and 4.6° F. In Table 9a one temperature reaction is shown in curve form. None of the healthy animals reacted to the "abortin." In comparing the results, here we note that in a herd of 18 animals, 100 per cent positive results were obtained by the intravenous method.

Conclusions.—In a small herd of 18 animals, in which abortion has been present for only a very short time, 100 per cent of positive results were obtained by the intravenous method.

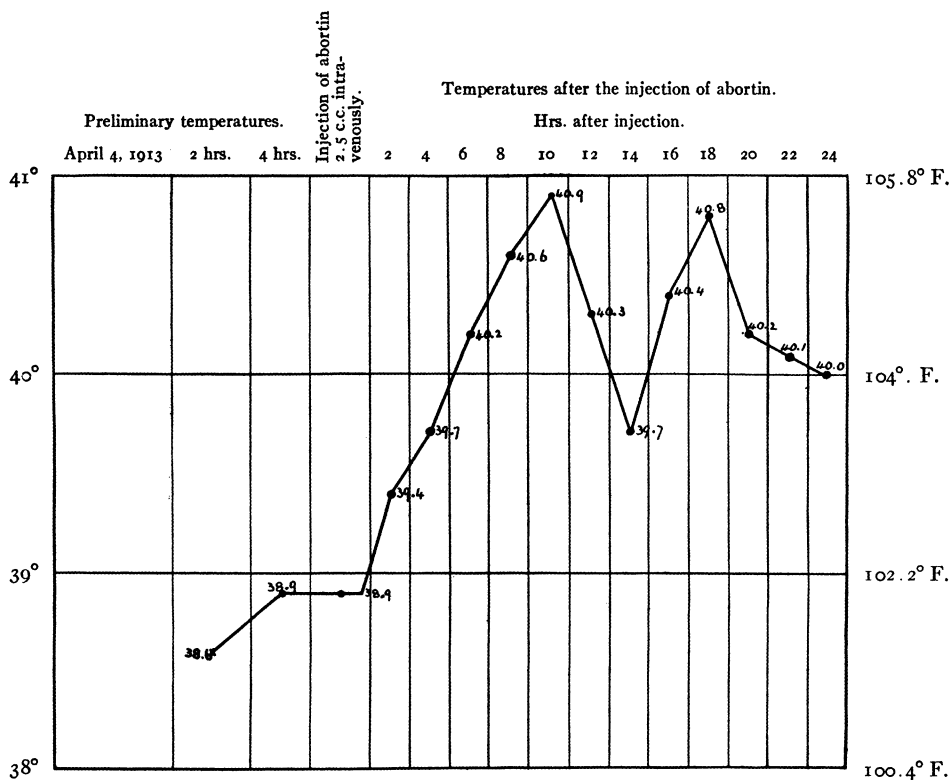
Herd No. 7.—The high percentage of failures in absolutely healthy animals which deprived the preceding "abortin" test of its specificity was thought to be perhaps overcome by purifying the "abortin," and for this reason a herd was tested with precipitated and "plain" concentrated "abortin," prepared as explained and discussed in the introduction. The herd selected for this purpose consisted of 69 animals, in which, according to complete records, 31 animals had aborted. The test was made on May 13, 1913, and the results are shown in Table 10.

TABLE 9.
HERD No. 6.

ANIMAL NUMBER	SERUM TESTS				INTER- PRETA- TION OF SERUM TESTS	TEMPERATURES AFTER INTRAVENOUS INJECTION OF 2.5 C.C. OF ABORTIN AT 9:00 P.M. APRIL 5, 1913										RESULT OF ABORTIN TEST	HISTORY
	1st Agglu- tination 2/25/13	1st Comple- ment Fix- ation	2d Agglu- tination 4/4/13	2d Comple- ment Fix- ation		6:00 P.M.	8:00 P.M.	12:15 A.M.	4:45 A.M.	6:15 A.M.	7:30 A.M.	9:30 A.M.	11:30 A.M.	12:30 P.M.	1:30 P.M.		
2.....	=0.02	=0.05	101.2	101.0	101.2	101.1	101.2	102.	102.3	101.5	101.	101.1	Heifer breed.
3.....	=0.05	=0.02	102.	102.8	101.8	101.6	101.6	102.3	101.8	101.3	101.2	101.8	
4.....	>0.01	>0.01	Positive	101.4	101.8	101.4	101.8	102.	102.8	102.1	102.1	101.6	101.8	Positive	
5.....	=0.02	=0.005	=0.01	=0.005	101.4	102.8	101.4	101.8	102.5	103.6	104.	103.5	102.8	102.2	A. 4/2/13.
6.....	=0.02	=0.02	101.	101.8	101.8	101.4	101.7	101.6	102.	101.	100.8	101.	
7.....	>0.01	=0.02	Positive	101.4	101.8	102.8	101.3	102.3	105.	105.6	104.2	103.3	104.6	Positive	
8.....	=0.01	=0.05	>0.01	=0.02	101.5	101.5	101.3	101.8	101.3	102.7	102.3	102.3	101.4	101.2	Heifer breed.
9.....	=0.05	=0.05	101.8	102.1	101.5	101.5	102.	102.3	102.3	102.3	101.3	101.4	
10.....	=0.05	=0.05	101.3	101.6	101.5	101.4	102.1	102.7	102.1	102.1	101.3	101.4	
11.....	=0.05	=0.02	101.6	101.2	102.2	101.4	102.1	104.8	104.4	105.2	101.3	101.8	Heifer breed.
12.....	>0.01	=0.02	>0.01	=0.02	Positive	101.1	101.2	101.4	102.2	102.5	102.1	102.4	105.2	101.8	101.2	Positive	
13.....	=0.01	=0.02	101.4	101.8	101.4	102.2	102.5	102.1	102.1	105.2	105.8	105.	
14.....	=0.02	=0.02	101.1	101.8	101.6	101.8	101.4	101.6	101.8	101.6	101.7	101.3	Heifer breed.
15.....	=0.01	=0.02	101.2	101.6	100.9	101.2	102.	101.4	101.8	102.8	102.3	101.8	
16.....	=0.01	=0.02	101.4	101.8	101.1	101.2	101.	101.8	102.2	102.2	102.3	101.6	
17.....	=0.05	=0.05	102.3	102.8	102.1	101.9	102.1	102.1	102.2	102.8	102.6	101.7	Heifer breed.
18.....	>0.01	=0.02	>0.01	=0.02	Positive	102.8	102.5	102.4	104.6	105.2	105.4	105.8	105.7	105.5	104.8	
19.....	>0.01	>0.01	102.	102.5	102.4	104.6	105.2	105.4	105.8	105.7	105.5	104.8	Positive	

According to the serum tests 26 animals (Nos. 7, 8, 11, 13, 17, 20, 21, 22, 26, 28, 35, 38, 43, 101, 103, 106, 110, 111, 113, 114, 124, D, B, C, E, A) were still showing immune bodies in the serum. Twenty animals (Nos. 4, 7, 11, 22, 23, 26, 28, 29, 35, 43, 44, 50, 57, 106, 111, D, B, C, E, A) reacted to the "abortin" test, out of which 5 animals (Nos. 4, 29, 44, 57, 50) were healthy. Only 15 serum reactors (Nos. 7, 11,

TABLE 9a.



Cow 8, Herd 6. Aborted April 2, 1913.

Abortin test, April 4, 1913.

Agglutination: <0.01.

Complement fixation: 0.02.

Highest temperature before injection 101.4° F.

Highest temperature after injection 105.6° F.

Difference in temperature 4.2° F.

Reaction: positive.

22, 23, 26, 28, 35, 43, 106, 111, D, B, C, E, A) actually reacted to the "abortin." In Table 10a one of the reactions is shown in curve form.

The temperature with the precipitated "abortin" showed differences between the preliminary and the test temperatures from 2.2° to 5.2° F., average 3.91° F. With

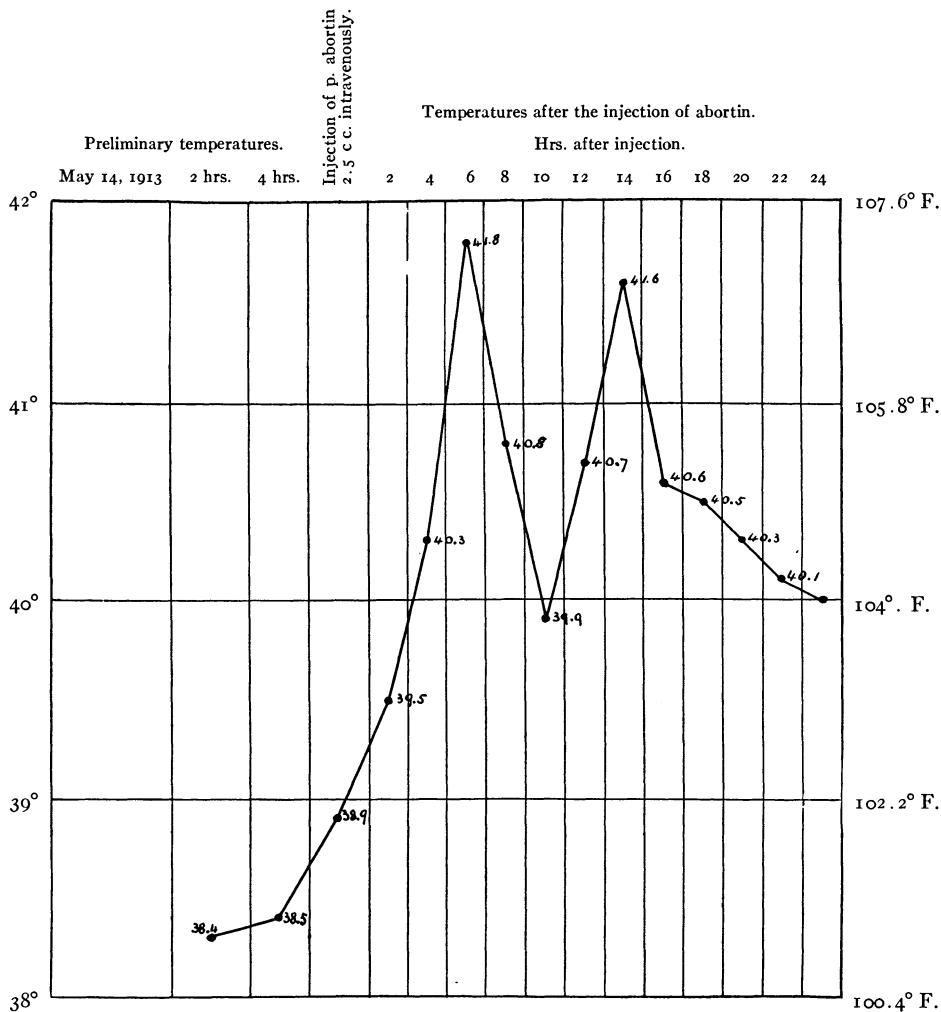
TABLE 10.
HERD No. 7.

ANIMAL NUMBER	AGE YEARS	SERUM TESTS				INTER- PRETA- TION OF SERUM TESTS	TEMPERA- TURES BEFORE INJECTION MAY 13, 1913		INJECTION	TEMPERATURES AFTER INJECTION MAY 14, 1913								
		1st Agglu- tination 7/10/12	1st Complement Fixation	2d Agglu- tination 4/21/13	2d Complement Fixation		4:00 P.M.	6:00 P.M.		Dose of Abortin at 7:00 P.M. Precipitated	12:00 P.M.	2:00 A.M.	4:00 A.M.	6:00 A.M.	8:00 A.M.	10:00 A.M.	12:00 N.	3 P.
2.....	6	=0.05	=0	=0.05	=0	101.4	101.4	2.5 c.c. intravenously	101.3	101.8	101.4	101.5	101.8	101.	101.	
3.....	10	=0.05	=0	=0.05	=0	101.2	101.2	"	101.2	101.9	101.6	101.6	101.	101.4	101.2	
4.....	8	=0.02	=0	=0	=0	101.	101.2	"	101.	101.2	102.	103.8	105.6	104.6	103.	
5.....	11	=0.05	=0	=0.05	=0	102.3	102.8	"	102.5	103.1	103.	102.	101.8	102.	101.8	
6.....	5	=0.02	=0.1	=0.05	=0	101.2	101.2	"	101.2	101.2	101.8	101.9	101.5	101.8	102.	
7.....	5	>0.01	=0.02	=0.01	=0	Positive	101.	101.5	"	102.2	101.6	102.2	102.5	103.2	102.2	104.8	
8.....	5	>0.01	=0.05	=0.05	=0.05	"	101.8	101.9	"	102.5	103.	104.8	105.8	102.8	101.3	103.3	
9.....	5	=0.02	=0	=0.02	=0	102.	101.8	"	
10.....	12	=0.05	=0	=0.05	=0	101.	101.2	"	101.7	101.2	101.3	101.8	101.8	100.8	101.2	
11.....	9	>0.01	=0.02	>0.01	=0.02	Positive	101.2	102.2	"	102.8	104.8	105.5	105.	104.9	104.9	105.	
12.....	9	=0.02	=0	=0.05	=0	101.3	102.1	"	101.8	101.8	102.	102.2	101.9	101.7	102.	
13.....	12	>0.01	=0.02	>0.01	=0.02	Positive	101.	101.2	"	101.7	101.2	101.4	101.8	101.8	101.8	101.8	
14.....	9	=0.02	=0	=0.01	=0	101.5	101.5	"	101.4	102.2	100.5	101.8	101.6	102.2	100.9	
15.....	7	=0.05	=0	=0	=0	101.1	101.2	"	101.7	102.2	102.2	102.8	103.2	102.2	103.4	
16.....	12	=0.02	=0	=0.05	=0	102.	101.8	"	101.4	101.8	102.1	102.	102.2	100.9	104.4	
17.....	12	=0.02	=0	>0.01	=0.005	Positive	102.	101.7	"	102.	102.	101.4	102.2	102.4	101.	102.3	
18.....	11	=0.02	=0	=0.05	=0	"	101.8	101.8	"	101.7	101.8	101.8	101.1	101.6	101.2	101.6	
19.....	6	=0.05	=0	=0.05	=0	"	101.5	102.4	"	103.2	102.	102.7	102.5	102.7	101.	103.	
20.....	5	>0.01	=0.005	>0.01	=0.02	Positive	102.4	102.1	"	102.2	102.	101.6	102.1	102.6	102.3	103.1	
21.....	4	=0.02	=0.05	=0.02	=0.02	"	101.8	102.1	"	101.2	101.8	101.8	102.4	102.	102.2	102.4	
22.....	6	=0.02	=0.05	=0.05	=0	"	101.5	101.3	"	101.6	103.	103.8	103.6	102.9	103.4	104.6	
23.....	6	=0.02	=0	=0.05	=0	102.	102.4	"	102.2	101.9	101.8	102.	101.8	101.9	101.6	
24.....	6	=0.02	=0	=0.02	=0	102.1	102.2	"	102.1	102.	102.	102.7	102.5	99.	101.	
25.....	6	=0.02	=0	=0.02	=0	101.3	101.3	"	101.8	101.8	101.4	101.8	101.8	101.8	101.8	
26.....	6	>0.01	=0.005	=0.02	=0.005	Positive	101.8	101.2	"	102.2	102.8	102.	104.	103.3	103.5	104.2	
28.....	4	>0.01	=0.005	>0.01	=0.005	"	101.8	101.8	"	105.2	106.	104.	102.4	104.2	103.5	105.2	
29.....	4	>0.01	=0	101.8	101.8	"	103.4	106.2	105.	102.3	102.4	102.1	105.2	
30.....	3	=0	=0	101.8	101.9	"	101.4	101.8	101.4	100.2	101.7	101.4	101.6	
34.....	4	=0.05	=0	=0.02	=0	101.2	101.	"	101.2	102.1	101.	101.3	101.6	101.2	102.6	
35.....	4	>0.01	=0.005	>0.01	=0.005	Positive	101.4	101.3	"	104.	105.8	105.	103.4	104.4	104.4	105.4	
36.....	3	=0.02	=0.1	=0.05	=0	101.	101.6	"	101.2	101.	101.2	102.	101.3	100.6	100.8	
37.....	3	=0.02	=0	=0.02	=0	101.5	101.6	"	101.	101.2	101.3	102.	101.8	101.4	101.8	
38.....	3	>0.01	=0.02	=0.05	=0.02	Positive	101.3	102.2	"	102.2	102.	101.6	101.9	102.3	101.6	101.8	
39.....	3	>0.05	=0	=0.05	=0	101.4	101.6	2.5 c.c. intravenously	101.1	101.5	101.8	102.1	101.7	100.8	101.2	
40.....	3	>0.05	=0	=0.05	=0	101.5	101.6	"	104.	101.6	101.2	102.	101.8	100.7	101.	
41.....	2	=0.02	=0	101.1	102.	"	102.	102.2	102.	102.	101.9	102.5	102.	
42.....	2	=0.05	=0	101.	101.6	"	101.8	101.8	101.8	102.1	101.9	101.3	101.8	
43.....	2	=0.02	=0.005	Positive	101.9	101.8	"	103.2	104.3	105.5	105.2	107.	104.5	105.8	
44.....	2	=0.05	=0	101.6	101.5	"	102.	104.4	106.	103.9	102.2	102.	101.5	
Heifer " 45	2	=0.01	=0	=0.02	=0	100.5	101.8	"	101.	101.2	101.3	102.	102.1	101.6	102.2	
" " 47	1 1/2	=0.05	=0	101.6	101.9	"	101.8	102.	101.8	101.9	101.9	101.9	101.9	
" " 48	2	=0.01	=0	=0.05	=0	101.8	102.	"	102.	102.	102.1	102.2	102.	102.	101.4	
49.....	2	=0	=0	101.6	102.2	"	101.8	102.	101.8	102.	101.8	101.9	101.6	
50.....	2	=0.02	=0	101.8	102.4	"	102.4	103.2	103.7	104.	105.1	105.2	105.8	
51.....	2	=0.05	=0	=0.05	=0	101.6	102.2	"	101.2	101.3	101.5	101.8	101.4	101.9	101.3	
52.....	1 1/2	=0.02	=0	=0.05	=0	101.6	101.4	"	101.8	101.2	101.5	101.8	101.3	101.6	101.3	
53.....	1 1/2	=0.02	=0	101.4	101.5	"	101.2	101.	101.	101.5	101.3	101.	101.	
54.....	1 1/2	=0.02	=0	=0.02	=0	101.2	101.4	"	101.8	101.8	102.4	102.4	102.4	102.	102.	
55.....	2	=0	=0	=0.02	=0	101.4	101.9	"	102.	102.8	102.	102.2	101.9	101.5	101.1	
56.....	1 1/2	=0.02	=0	=0	=0	101.5	101.6	"	102.	102.7	102.2	102.	102.2	101.6	101.3	
57.....	2	=0	=0	101.8	101.8	"	101.8	101.6	102.5	102.8	103.2	104.	104.6	
58.....	2	=0	=0	=0	=0	101.7	101.8	"	101.8	101.4	101.4	101.8	101.8	101.4	103.	
60.....	3	=0.05	=0	=0	=0	100.7	100.6	"	102.5	102.8	102.6	102.9	102.6	102.3	102.	
101.....	11	=0.02	=0.05	=0.02	=0.05	Positive	101.4	101.8	"	102.	102.	101.8	101.8	101.6	101.6	101.9	
102.....	9	=0.02	=0	=0.02	=0	100.6	101.4	"	101.8	101.4	101.3	101.	101.	101.8	100.8	
103.....	9	=0.05	=0.05	=0.02	=0.05	Positive	100.2	101.	"	101.	101.2	101.5	101.3	101.4	101.2	101.	
106.....	5	>0.01	=0.01	=0.05	=0.05	"	101.6	102.	"	102.	101.8	102.2	102.6	103.3	104.4	105.2	
110.....	5	>0.01	=0.02	=0.05	=0.005	"	101.8	101.6	"	101.4	102.2	102.1	102.	102.1	102.2	101.8	
111.....	6	>0.01	=0.005	>0.01	=0.005	"	101.4	102.4	"	102.	102.	102.6	104.2	105.2	105.9	104.5	
112.....	15	=0.02	=0	=0	=0	101.2	101.2	"	102.2	102.2	102.	101.8	101.8	101.	101.2	
113.....	14	=0.02	=0	>0.01	=0.005	Positive	101.8	101.9	"	102.	101.9	101.8	102.2	101.9	102.	102.	
114.....	3	>0.01	=0.005	>0.01	=0.005	"	101.8	102.1	"	101.2	101.9	101.7	102.8	102.8	101.8	102.	
124.....	3	=0.01	=0.005	>0.01	=0.005	"	101.4	102.2	"	101.8	101.1	102.	101.6	103.3	101.	101.5	
125.....	4	>0.01	=0.1	=0.05	=0	101.6	102.2	"	101.8	101.9	101.6	101.9	101.8	101.	100.8	
20.....	4	=0.02	=0	=0.05	=0	101.2	101.8	"	102.	102.2	101.8	102.1	102.1	100.6	100.8	
D.....	4	>0.01	=0.02	>0.01	=0.005	Positive	101.	102.	2.5 c.c. intravenously	104.4	107.2	105.4	103.8	105.5	106.8	105.3	101.	
B.....	2	>0.01	=0.005	>0.01	=0.02	"	101.8	101.8	"	101.9	102.2	102.1	102.4	105.	105.8	105.3	101.	
C.....	4	>0.01	=0.02	=0.02	=0.02	"	101.4	101.7	"	102.1	101.4	101.6	102.	104.8	106.	105.1	
E.....	2	=0.01	=0.005	"	101.8	101.2	"	106.6	105.2	104.8	104.4					

TABLE 10.
HERD No. 7.

TEMPERATURES BEFORE INJECTION MAY 13, 1913		INJECTION	TEMPERATURES AFTER INJECTION MAY 14, 1913										RESULT OF THE ABORTIN TEST	HISTORY
4:00 P.M.	6:00 P.M.		Dose of Abortin at 7:00 P.M. Precipitated											
			12:00 P.M.	2:00 A.M.	4:00 A.M.	6:00 A.M.	8:00 A.M.	10:00 A.M.	12:00 N.	3:00 P.M.	5:00 P.M.			
101.4	101.4	2.5 c.c. intravenously	101.3	101.8	101.4	101.5	101.8	101.	101.				Aborted, 7/24/09; calved, 8/27/12 (calf died); Granular Vaginitis; difficult to breed.	
101.2	101.2	"	101.2	101.9	101.6	101.6	101.	101.4	101.2			Positive	Calved, 3/23/12; difficult to breed.	
101.	101.2	"	101.	101.2	102.	103.8	105.6	104.6	103.	100.9			" 2/28/13.	
102.3	102.8	"	102.5	103.1	103.	102.	101.8	102.	101.8				" 7/9/12; due to calf 6/20/13.	
101.2	101.2	"	101.2	101.2	101.8	101.9	101.5	101.8	102.				Aborted, 7/4/09; calved, 12/20/12.	
101.	101.5	"	102.2	101.6	102.2	102.5	103.2	102.2	104.8	102.		Positive	" 8/7/09; last calf, 3/7/13.	
101.8	101.9	"	102.5	103.	104.8	105.8	102.8	101.3	103.3	103.			Never aborted; due 7/10/13; last calf, 6/29/12.	
102.	101.8	"											Not tested, due to calf 6/9; last calf, 3/8/12.	
101.	101.2	"	101.7	101.2	101.3	101.8	101.8	100.8	101.2				Calved, 11/28/12.	
101.2	102.2	"	102.8	104.8	105.5	105.	104.9	104.9	105.	104.3		Positive	Aborted, 4/6/08; calved, 3/7/09 (calf died); last calf, 9/30/12.	
101.3	102.1	"	101.8	101.8	102.	102.2	101.9	101.7	102.				Aborted, 3/6/07; last calf, 8/1/12.	
101.2	101.2	"	101.8	101.2	101.4	102.6	101.8	101.3	101.8				Calved, 11/28/12; calved, 3/12/11.	
101.2	102.2	"	101.8	104.8	105.5	105.	104.9	104.9	105.	104.3		Positive	Aborted, 4/6/08; calved, 3/7/09 (calf died); last calf, 9/30/12.	
101.3	102.1	"	101.8	101.8	102.	102.2	101.9	101.7	102.				Aborted, 3/6/07; last calf, 8/1/12.	
101.2	101.7	"	101.8	101.6	104.1	102.	102.	101.3	101.8				" 11/8/08; calved, 3/12/13.	
101.5	101.5	"	101.4	102.2	100.5	101.8	101.6	102.	100.9				" 10/14/07; calved, 12/21/12.	
101.1	101.2	"	101.7	102.2	102.8	102.3	102.2	102.4	103.2				Calved, 3/18/13.	
102.	101.8	"	101.4	101.8	102.1	102.	102.2	100.9	101.4				Aborted, 3/24/08; last calf, 3/14/13.	
102.	101.7	"	102.	102.	104.2	102.4	101.	102.3					Last calf, 7/17/12.	
101.8	101.8	"	101.7	101.8	101.8	101.1	101.6	101.2	101.6				Calved, 2/12/13.	
101.5	102.4	"	103.2	102.	102.7	102.5	102.7	101.	103.	102.3			Aborted, 10/7/09; last calved, 3/15/13.	
102.4	102.1	"	102.2	102.	101.6	102.1	102.6	102.3	103.1	103.3			Calved, 5/12/12; difficult to breed.	
101.8	102.1	"	101.2	101.8	101.8	102.4	102.	102.2	102.4	103.	104.4	Positive	Calved, 7/18/12.	
101.5	101.3	"	101.6	103.	103.8	103.6	102.9	103.4	104.6	103.1		"	Aborted, 1/19/10; calved, 12/29/12.	
102.	102.4	"	102.2	101.9	101.8	102.	101.8	101.9	101.6				" 9/10/09; calved, 9/14/12 (calf died).	
102.1	102.2	"	102.1	102.	102.	102.7	102.5	99.	101.				Aborted 10/7/08; calved, 4/21/13.	
101.3	101.3	"	101.8	101.8	101.4	101.6	101.8	101.8	101.8				" 4/28/09; calved, 12/7/12.	
101.8	101.2	"	102.2	102.8	102.	104.	103.3	103.5	104.2			Positive	Never aborted; calved, 9/3/12 (calf died).	
101.8	101.8	"	105.2	106.	104.	102.4	104.2	103.5	105.2			"	Aborted, 7/19/11; calved, 9/9/12 (4 months delivered).	
101.8	101.8	"	103.4	106.2	105.	102.3	102.4	102.1	105.2	102.6		Positive	Aborted, 8/4/11; calved, 9/17/12.	
101.8	101.9	"	101.4	101.8	101.4	100.2	101.7	101.4	101.6				Calved, 1/13/13.	
101.2	101.	"	101.2	101.2	101.	101.3	101.6	102.	102.6				Last calved, 3/13/13.	
101.4	101.3	"	104.	105.8	105.	103.4	104.4	104.4	104.	104.		Positive	Aborted, 7/24/11 and 10/24/10; last calved, 7/1/12.	
101.	101.6	"	101.2	101.	101.2	102.	101.3	100.6	100.8				Calved, 7/16/12.	
101.5	101.6	Ordinary	101.	101.2	101.3	102.	101.8	101.4	101.				7/28/12.	
101.3	102.2	"	102.2	102.	101.6	101.9	102.3	101.6	101.8				Due to calf, 6/20/13.	
101.4	101.6	2.5 c.c. intravenously	101.1	101.5	101.8	102.1	101.7	100.8	101.2				Aborted, 4/15/11; due 6/25/13—calved, 5/14/12.	
101.5	101.6	"	104.	101.6	101.2	102.	101.8	100.7	101.				Calved, 5/6/12 (7/5/13).	
101.1	102.	"	102.	102.2	102.	102.	101.9	102.5	102.				" 12/19/12 (1st).	
101.	101.6	"	101.8	101.8	101.8	102.1	101.9	101.3	101.8				" 12/11/12 (1st).	
101.9	101.8	"	103.2	104.3	105.5	105.2	107.	104.5	105.8	105.6		Positive	Never aborted; calved, 1/26/13 (calf died) (1st).	
101.6	101.5	"	102.	104.4	106.	103.9	102.2	102.	101.5			"	Never calved (due July).	
100.5	101.8	"	101.	101.2	101.3	102.	102.1	101.6	102.2				" " "	
101.6	101.9	"	101.8	102.	101.8	101.9	101.9	102.	101.9				" " "	
101.8	102.	"	102.	102.1	102.2	102.	102.	102.	101.4				" " "	
101.6	102.2	"	101.8	102.	101.8	102.	101.8	101.9	101.6				" " "	
101.8	102.4	"	102.4	103.2	103.7	104.	105.1	105.2	105.8	105.3		Positive	" " "	
101.6	102.2	"	101.2	101.3	101.5	101.8	101.4	101.9	101.3				" " "	
101.6	102.4	"	101.8	101.2	101.5	101.5	101.8	101.3	101.6				" " "	
101.4	101.5	"	101.2	101.	101.	101.5	101.3	101.	101.				" " "	
101.2	101.4	"	101.8	101.8	102.4	102.4	102.4	102.	102.				" " "	
101.4	101.9	"	102.	102.8	102.	102.2	101.9	101.5	101.1				" " "	
101.5	101.6	"	102.	102.7	102.2	102.	102.2	101.6	101.3				" " "	
101.8	101.8	"	101.8	101.6	102.5	102.8	103.2	104.	104.6	104.		Positive	Due to calf, 7/5/13.	
101.7	101.8	"	101.8	101.4	101.4	101.8	101.8	101.4	103.	101.5			Not yet calved.	
100.7	100.6	"	102.5	102.8	102.6	102.9	102.6	102.3	102.	101.5			Calved, 7/28/12.	
101.4	101.8	"	102.	102.	101.8	101.8	101.6	101.6	101.9				" 9/12/12.	
100.6	101.4	"	101.8	101.4	101.3	101.	101.	101.8	100.8				Aborted, 6/7/09; calved, 1/14/13.	
100.2	101.	"	101.	101.2	101.5	101.3	101.4	101.2	101.				" 8/6/09; " 11/11/12.	
101.6	102.	"	102.	101.8	102.2	102.6	103.3	104.4	105.2	104.8		Positive	" 8/1/10; " 11/29/12.	
101.8	101.6	"	101.4	102.2	102.1	102.	102.1	102.2	101.8				" 11/18/11; " 11/10/12.	
101.4	102.4	"	102.	102.	102.6	104.2	105.2	105.9	104.5	104.2		Positive	Never aborted; calved, 6/20/13 — 6/9/12.	
101.2	101.2	"	102.2	102.2	102.	101.8	101.8	101.	101.2				Aborted, 6/8/09; calved, 9/7/12.	
101.8	101.9	"	102.	102.9	101.8	102.2	101.9	102.	102.				" 7/21/12; " 6/10/13.	
101.8	101.2	"	101.2	101.9	101.7	102.8	102.7	101.8	102.				" 9/10/11; " 1912.	
101.4	102.2	"	101.8	102.1	102.	101.6	103.3	101.	101.5				" 9/23/11; " 12/ 3/12.	
101.6	102.2	"	101.8	101.9	101.6	101.9	101.8	101.	101.2				" 6/ 8/11; " 9/ 7/12.	
101.2	101.8	"	102.	102.2	101.8	102.1	102.1	100.6	100.8				Calved, 4/10/13.	
101.	102.	Precipitated	104.4	107.2	105.4	103.8	105.5	106.8	105.3	105.		Positive	Aborted, 7/21/12; (8-month calf).	
101.8	101.8	2.5 c.c. intravenously	101.9	102.2	102.1	102.4	105.	105.8	105.3	104.8		"	" 6/ 9/12.	
101.4	101.7	"	101.2	101.4	101.6	102.	105.8	105.	104.6			"	" 6/13/12.	
101.8	101.2	"	106.6	105.2	104.8	104.4	104.2	106.7	105.1	106.4		"	" March, 1913.	
101.2	101.6	"	102.6	105.4	105.2	103.6	105.4	106.6	104.6	103.2		"	" 2/10/13 (calf negative).	

TABLE 10a.



Cow D, Herd 7. Aborted July 2, 1912.
 Abortin test May 14, 1913; Serum tests May 14, 1913.
 Agglutination: <0.01.
 Complement fixation: <0.005.
 Highest temperature before injection 102.0° F.
 Highest temperature after injection 107.2° F.
 Difference in temperature 5.2° F.
 Reaction: positive.

the plain "abortin," differences from 2.8° to 5.1° F., an average of 3.76° F., were recorded. Comparing the results obtained with the ordinary and the precipitated "abortin" in this herd by the intravenous method, the following interesting results, as shown in Table 11, are of value.

TABLE 11.
COMPARISON OF ORDINARY WITH PRECIPITATED "ABORTIN" TEST IN THE SAME HERD.
Application: Intravenously.

PLAIN "ABORTIN": 33 ANIMALS				PRECIPITATED "ABORTIN": 36 ANIMALS			
Aborted for the Last Five Years	Reacted to Serum Tests	Aborted in Last Two Years	Reacted to Abortin	Aborted for the Last Five Years	Reacted to Serum Tests	Aborted in Last Two Years	Reacted to Abortin
9	10	4	3, or 30 per cent (6, or 60 per cent)	21	16	14	14, or 87.5 per cent
Out of 20 healthy animals reacted: 3, or 15 per cent				Out of 20 healthy animals: 2, or 10 per cent			

By means of the precipitated "abortin," out of 36 animals 87.5 per cent were diagnosed correctly, with the "plain abortin" only 30 per cent were detected to be affected with *B. abortus*. Each lot of the non-infected animals inoculated with "plain" or "precipitated abortin," respectively, showed the following differences: The plain "abortin" gave 15 per cent failures; the precipitated "abortin" gave only 10 per cent failures in non-infected animals or bovines which did not show any immune bodies in the blood serum.

Conclusions.—In a herd of 69 animals, by means of the precipitated "abortin" and the intravenous method, 87.5 per cent of the animals infected with *B. abortus* could be diagnosed. In using a "purified abortin," the "non-specific reactions" can, to a certain extent, be reduced, but it has to be proven by further tests if this observation is a constant one. So far it does not seem possible to eliminate this non-specificity of the reaction.

APPENDIX.

Herd No. 3.—As explained above, "abortin" is distributed commercially, and it was considered advisable to test this preparation for its practical value. It can be mentioned here that this commercial preparation had no antigenic properties whatever, neither by animal inoculations nor by using it as an antigen in the complement fixation test. A herd, consisting of 61 animals, in which infectious abortion had existed for the last four years, was injected with this preparation, according to the instructions given by the manufacturer, the dose being 4 c.c. subcutaneously. The laboratory number of this preparation was 8815, the date of preparation was June 15, 1912. The results obtained are shown in Table 12.

Conclusions.—The commercially prepared and distributed "abortin" solution cannot be recommended for tests, because it has no antigenic properties, and the non-specificity of the preparation is also evident.

Conjunctival tests.—All the animals of Herd No. 7 were also tested by the "ophthalmic test," 2-4 drops of a 5 per cent solution of precipitated "abortin" in saline solution were instilled into the conjunctival sac as customary for such tests. The readings, made from the tenth hour on, revealed no reactions.

Conclusions.—The conjunctival test cannot be used for the diagnosis of infectious abortion.

Summary of conclusions.—In comparing the result obtained by Belfanti, and Zwick and Zeller with ours (see Table 13), we cannot blame these investigators for condemning the "abortin," as the results are certainly not encouraging. Still we do not take such a skeptical view concerning this preparation.

From the above stated experiments with "abortin" on 209 animals with our own preparations, we learned that the product permits a conclusive diagnosis in 59.3 per cent of the cases of infection, if the serum reaction is taken in consideration for comparison. In healthy animals about 29.1 per cent failures are recorded, or only 70.9 per cent of the "abortin" reactions are supported by positive serum reaction. The reactions are mostly typical, but only when a "purified precipitated abortin" is used. The failures in the healthy animals can be reduced as far as 10 per cent when this preparation is used. The injections of the biologic product should always be made intravenously; the dangers of general anaphylactic symptoms are very small in number. In recently infected herds the results are better than in old infected ones. In several instances, also, aborters reacted to the "abortin" test when there were no immune bodies in the serum of the tested animals.

We cannot support the view of Giltner that the "abortin" has a certain immunizatoric effect against natural infection, as some heifers injected with our best "abortin" have since become infected and have aborted.

SUMMARY.

The serum tests, agglutination and complement fixation, are the most reliable methods to determine the existence of infectious abortion in a herd, and to detect the bovines which are or have been infected with *B. abortus*.

The "abortin" test in the form and with the preparation recommended by the English Commission is unreliable and misleading. Encouraging results are obtained with a precipitated

TABLE 12.

HERD NO. 3.

ANIMAL NUMBER	SERUM TESTS				INTER- PRETA- TION OF SERUM TESTS	TEMPERATURES BEFORE INJECTION AUG. 15, 1912					TEMPERATURES AFTER SUBCUTANEOUS INJECTION OF 4 c.c. OF COMMERCIAL ABORTIN AT 6:00 P.M. AUG. 15 AND 16, 1912								RESULT OF THE ABORTIN TEST	HISTORY
	1st Agglu- tination 8/16/12	1st Comple- ment Fixation	2d Agglu- tination 1/12/13	2d Comple- ment Fixation		1:00 P.M.	3:00 P.M.	4:00 P.M.	5:00 P.M.	7:00 P.M.	9:00 P.M.	11:00 P.M.	1:00 A.M.	3:00 A.M.	5:00 A.M.	7:00 A.M.	9:00 A.M.	11:00 A.M.		
1.	...	=0.1	...	=0.2	...	102.6	103.	103.8	104.	104.6	102.7	102.3	103.3	103.	102.	100.3	100.2	100.3	...	Aborted, 1/19/12.
2.	101.7	101.	101.5	101.8	101.8	100.6	99.1	99.7	100.	100.	100.	100.	99.2	...	
3.	101.2	101.9	101.8	101.8	102.4	101.8	101.	100.7	100.5	101.	101.	101.	101.2	...	
4.	=0	...	101.	101.8	101.2	101.	101.8	100.2	100.7	100.9	103.	100.9	101.3	101.2	100.9	...	
5.	...	=0.1	101.	101.8	101.2	101.	101.3	101.8	101.	100.8	100.7	100.5	100.8	101.	100.6	...	1 premature calf. No abortions for last 4 years. No abortion. " "
6.	101.6	101.1	100.9	101.1	101.5	100.5	100.3	100.9	100.	100.4	101.1	100.2	100.4	...	
7.	...	=0.02	>0.01	=0.02	Positive	101.2	101.3	101.1	101.6	101.5	101.5	100.5	101.1	100.5	100.8	101.4	101.2	100.8	...	
8.	...	=0.05	=0.01	=0	"	101.2	101.2	101.8	101.4	102.2	101.3	100.6	100.5	101.5	100.9	101.	101.2	100.8	...	
9.	...	=0.02	=0.05	=0.005	"	101.1	101.2	101.7	101.4	102.1	101.6	101.	100.6	100.5	101.3	100.9	101.	100.9	...	19/9/1911 A. No abortion. " "
10.	101.6	102.	102.4	102.	102.8	102.6	99.1	100.	100.	100.7	100.5	101.2	101.	...	
11.	101.4	101.8	102.4	101.8	102.6	102.3	100.9	100.9	101.1	100.7	101.	101.2	101.	...	
12.	101.1	101.5	101.8	101.1	101.6	102.2	101.3	102.	101.	101.	100.9	101.3	101.3	101.	
13.	101.3	101.2	101.2	101.1	102.1	102.2	101.3	102.	101.	101.	100.9	101.3	101.5	...	Aborted once. No history. Never aborted. " "
14.	101.8	102.2	102.	102.2	101.3	101.4	101.7	101.7	101.	101.5	100.8	100.9	101.5	...	
15.	...	=0.005	=0.02	=0	Positive	101.6	101.	101.5	100.6	101.5	101.8	101.	100.2	100.7	100.7	101.	101.	100.5	...	
16.	...	=0.005	=0.02	=0	"	101.6	101.4	101.4	101.6	102.1	101.9	100.8	101.8	101.6	100.9	101.4	101.7	100.8	...	
17.	101.6	101.4	101.1	101.6	102.4	101.5	100.1	101.	100.8	100.5	101.	101.1	100.5	...	No abortion. " "
18.	101.2	101.4	101.4	101.	101.8	101.2	101.1	101.2	100.9	100.	101.3	100.6	101.2	...	
19.	...	=0.05	=0.05	=0	Positive	101.7	102.	102.	102.	101.7	102.	101.1	101.2	101.5	101.	101.	101.2	101.2	...	
20.	103.4	101.	101.7	101.3	102.4	101.5	100.6	100.6	100.4	101.	100.9	100.6	100.9	...	
21.	...	=0.05	=0.05	=0	...	102.8	101.	101.5	101.3	102.	101.5	101.2	101.2	101.5	100.4	101.	100.8	100.7	...	Aborted twice. No abortion. " "
22.	103.4	101.	101.7	101.3	102.4	101.5	100.6	100.6	100.4	101.	100.9	100.6	100.9	...	
23.	...	=0.005	=0.02	=0	Positive	101.8	101.	101.5	101.3	102.4	101.5	100.6	100.6	100.4	101.	100.8	100.4	100.6	...	
24.	...	=0.005	=0.02	=0	...	101.3	102.6	101.6	101.6	102.4	101.9	100.8	100.5	99.8	100.4	101.	100.2	100.6	...	
25.	102.3	102.	102.6	102.4	102.5	101.3	100.4	101.	100.4	100.8	101.	100.8	100.7	...	Positive
26.	102.3	102.	102.6	102.4	102.6	101.9	102.4	102.	101.	100.4	101.	100.8	100.7	...	
27.	102.3	102.	102.6	102.4	102.6	101.9	102.4	102.	101.	100.4	101.	100.8	100.7	...	
28.	102.3	102.	102.6	102.4	102.6	101.9	102.4	102.	101.	100.4	101.	100.8	100.7	...	
29.	102.3	102.	102.6	102.4	102.6	101.9	102.4	102.	101.	100.4	101.	100.8	100.7	...	" "
30.	102.3	102.	102.6	102.4	102.6	101.9	102.4	102.	101.	100.4	101.	100.8	100.7	...	
31.	102.3	102.	102.6	102.4	102.6	101.9	102.4	102.	101.	100.4	101.	100.8	100.7	...	
32.	102.3	102.	102.6	102.4	102.6	101.9	102.4	102.	101.	100.4	101.	100.8	100.7	...	
33.	...	=0.05	=0.05	=0.02	Positive	102.5	102.6	102.4	102.8	102.6	100.1	100.2	101.6	101.3	101.	100.5	100.6	101.2	...	Positive
34.	...	=0.05	=0.05	=0.05	"	101.3	101.8	101.8	101.6	102.1	100.6	101.2	101.1	101.1	100.5	101.	100.1	100.1	...	

purified "abortin" by intravenous application. The reaction is not absolutely specific as a high percentage of healthy animals react to the injection of "abortin" products. This non-specificity is more frequently observed with an ordinary plain "abortin" than with our purified product.

By means of the "abortin" test we cannot decide whether an animal has been recently infected and will abort, or whether it is recovering from an invasion with *B. abortus*.

TABLE 1.

NO. OF HERDS	NO. OF ANIMALS IN HERD	NO. OF ABORTERS		TOTAL NO. OF REACTORS TO SERUM TEST		NO. OF REACTORS ABORTING TO SERUM TEST		NO. OF PREMATURE CALVES		NO. OF ANIMALS DIFFICULT TO BREED		REACTORS BUT NOT ABORTED.		NO. OF ANIMALS REACTED TO ABORTIN EITHER WAY	
			Per-centage		Per-centage		Per-centage		Per-centage		Per-centage		Per-centage		Per-centage
Herd No. 1	49	12	24.5	23	46.9	10	43.5	2	4	2	4	13	56.5	12	26.5
Herd No. 2	19	2	10.5	3	15.7	2	66.6	1	33.3	4	21.8
Herd No. 4	32	12	37.5	15	46.8	11	73.3	4	26.3	7	21.8
Herd No. 5	22(138)	14	63.6	14	63.6	12	85.7	1	4.5	2	14.3	13	59
Herd No. 6	18	1	5.5	4	22.2	1	25	3	75	4	22.2
Herd No. 7	69	31	44.9	26	37.6	17	65.4	9	34.6	20	28.9
Total	209	72	31	85	38.8	53	59.7	2	4	3	4.25	32	40	61	30.1
Commercial															
(Herd 3)	61	7	11.4	9	14.7	3	33.3	1	1.6	6	66.6	2	3.49
Belfanti	19	2	10.5	10	52.6	36.8
Zwick and	5	4	80	4	80	4	100	1	25	0	0
Zeller*	39	8	20.5	24	61.5	7	29.1	17	70.9	3	7.6

* Selection from the report made by the writers, *op. cit.*, p. 105.

TABLE I.

	REACTORS BUT NOT ABORTED.		NO. OF ANIMALS REACTED TO ABORTIN EITHER WAY		NUMBERS OF REACTORS TO ABORTIN			NO. OF ABORTERS REACTED TO ABORTIN COMPARED WITH ABORTUS		NO. OF HEALTHY OUT OF REACTORS		NUMBER REACTED			INJECTED	
						Compared with Serum Reactors	Compared with Abortin Reactors					Subcutaneously	Intravenously	Precipitated	Subcutaneously	Intravenously
		Percentage		Percentage		Percentage	Percentage		Percentage		Percentage					
13	56.5		12	26.5	6	26.0	46.1	3	23	7	53.8	13			49	
1	33.3		4	21.8	2	66.6	50.0	2	50	2	50	4			19	
4	26.3		7	21.8	0	40.0	85.5	5	71.4	1	14.2	2	5		12	20
2	14.3		13	59	9	65.0	69.2	8	61.5	4	30.7	3	10		10	12
3	75		4	22.2	4	100	100	1	25	0	0		4			18
9	34.6		20	28.9	15	57.6	75	12	60	5	25		20	14		69
32	40		61	30.1	42	59.3	70.9	31	48.4	19	29.1	22	39	14	90	119
6	66.6		2	3.49												
				36.8	7	70	70			12	100	2			01	
1	25		0	0	0	0	0		10.5		33.3					
17	70.9		3	7.6	2	8.3	66.6	0	0	1	33.3					